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Activation of Phosphoinositide Metabolism by Cholinergic Agents

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The primary acute, toxic effect of cholinergic agonists in the central nervous system is seizures. One system activated by cholinergic agonists is the hydrolysis of phosphoinositides (PI), a major site of action of lithium which potentiates convulsions associated with cholinergic agonists. Our goal was to determine how PI hydrolysis is affected by seizures and is modulated, especially by excitatory amino acids (EAA) which mediate brain damage.

Modulation of PI hydrolysis was due to a specific EAA receptor activated by quisqualate. Two major effects of quisqualate were identified, activation by itself and inhibition of the effects of other neurotransmitters, especially norepinephrine. Several agents affected these responses, notably calcium and sodium. An inhibitory amino acid had effects generally opposite to quisqualate. Seizures caused changes similar to those of EAA; a selective impairment of norepinephrine-induced PI hydrolysis. Seizures also increased PI hydrolysis mediated by cholinergic receptors. Thus, seizures increased the effects of stimulatory systems (cholinergic, EAA) and reduced the effects of the inhibitory norepinephrine system. The activity of protein kinase C was unaltered by seizures but tyrosine kinase activity was increased.

Thus, cholinergic agonist-induced seizures cause major alterations of the important second messenger-generating system of PI hydrolysis, mediated in part by EAA which in turn are influenced by a number of factors, most notably calcium.

Cholinergic agonist-induced seizures; Brain second messenger systems; Neurotransmitter/Neuromodulator interactions; RAV; Lab animals, Rats; Convulsions; Metabolism; CNS

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INTRODUCTION

Cholinergic agonist-induced seizures

In the mammalian central nervous system the major acute toxic effect of high doses of inhibitors of acetylcholinesterase is the stimulation of convulsions. This effect is due to accumulation of excessively high concentrations of the neurotransmitter acetylcholine that build up when acetylcholinesterase is inhibited, and the subsequent large amount of stimulation of cholinergic receptors (1). Seizures can also be produced by administration of direct-acting cholinergic agonists which mimic the action of acetylcholine on cholinergic receptors. Cholinergic agonists which are charged cannot cross the blood-brain barrier to enter the central nervous system (CNS) if they are administered peripherally, but central administration of such drugs (e.g., carbachol) induces seizures (2,3). In addition, central administration of carbachol is used widely to induce the important "kindling" model of seizures (4). Cholinergic agonists that are not charged, such as pilocarpine, can cross the blood-brain barrier and can cause seizures when administered either peripherally or centrally (5). Such seizures are often lethal, even when peripheral cholinergic effects are blocked by an antagonist, such as methylatropine, indicating that the centrally induced convulsant effects are sufficient to cause death. Pretreatment with a cholinergic antagonist that enters the brain (e.g., atropine) prevents both seizures and death (5), confirming that these are mediated by cholinergic receptors.

The mechanisms whereby cholinergic receptor stimulation leads to seizures and death are still not clear. Direct neurochemical studies with anticholinesterase drugs or cholinergic agonists are impaired by the great interindividual variation in the responses to these agents (6). Thus, a single dose of one of these agents can induce rapid seizures and death, prolonged seizures, or no seizure response in apparently identical groups of subjects, such as a single strain of rats. The use of lithium in conjunction with cholinergic agonists has provided in two ways an advantageous system to study: this cotreatment regimen provides a very reproducible convulsant response, resulting in all rats demonstrating seizures over very similar time courses, and the fact that lithium potentiates cholinergic agonist-induced seizures suggests that lithium affects an

important biochemical system mediating this response, thus providing a lead as to the biochemical basis of the lethal seizures.

Lithium potentiates responses to cholinergic agonists

Lithium was first reported to potentiate the convulsions and lethality of physostigmine (7,8). These studies used an acute dose of LiCl given several hours before administration of a normally subconvulsive dose of physostigmine. Honchar et al. (9) extended this work by reporting that lithium also had a proconvulsant effect on the responses to two cholinergic agonists, pilocarpine and arecoline. They also reported (9) that administration of lithium and cholinergic agonists resulted in large increases in the brain concentration of inositol monophosphate (IP^1), a metabolite produced from hydrolysis of inositol-containing lipids (phosphoinositides), an important receptor-coupled second messenger generating system (10).

Our laboratory extended this previous work in a number of reports (11-17). We utilized EEG recordings from electrodes implanted in specific regions of rat brains to identify seizures induced by pilocarpine, arecoline or physostigmine, each in the absence and presence of pretreatment with LiCl. Complete dose-response curves for each of these was reported (15). The seizures induced by coadministration of LiCl and cholinergic agonists appear identical with those induced by high doses of cholinergic agonists alone using both EEG and behavioral criteria, except that the utilization of LiCl plus a cholinergic agonist produces extremely consistent and reproducible seizure responses in contrast to the variability obtained with the use of cholinergic agonists alone. The seizures are manifested as generalized convulsive status epilepticus, the seizures appear approximately simultaneously in all brain regions that we have tested, and status epilepticus continues without abatement for several hours, until death occurs. We have also reported that cortical and hippocampal concentrations of acetylcholine are increased to unprecedented levels (higher even than after administration of soman) in rats treated with lithium and pilocarpine even though acetylcholinesterase was not inhibited (14). Such a massive accumulation of acetylcholine after treatment with lithium and pilocarpine emphasizes the important alterations occurring in the cholinergic system in response to this treatment. Two

papers have been published which report that lithium treatment potentiates the convulsant effect of two other anticholinesterases, paraoxon and soman (18,19). Increased brain levels of IP^1 were reported with these treatments (18,19) as had previously been reported with coadministration of lithium and other cholinergic agonists (9). The seizure-potentiating effect of lithium is, as far as we know, selective for cholinergic agonists because screening of several other convulsants showed little or no influence of lithium treatment (17). Thus, lithium is affecting a critical regulatory site that is specifically important in modulating the response to cholinergic stimuli. Identification of the initiating mechanisms should be extremely useful in the development of drugs to block this toxic, lethal response to cholinergic agonists. Examination of many of the classical anticonvulsants after lithium and pilocarpine treatment revealed that once seizures began, these drugs generally had little or no anticonvulsive effects (13). Thus, lithium has the interesting effect of selectivity potentiating the seizurogenic effects of all tested cholinergic agonists, and once seizures begin, they are difficult to control. Therefore, identification of the effects of lithium on the biochemical response to cholinergic agonists should be indicative of the biochemical mechanisms responsible for seizures initiated by cholinergic agonists.

Modulation of receptor-coupled second messenger systems

For receptor stimulation to induce a cellular response, the receptor must be coupled to a system that alters the intracellular biochemistry of neurons. During the last few years, it has become obvious that a major second messenger system in brain and many other tissues is the receptor-coupled hydrolysis of phosphoinositides. Several recent reviews have covered this in detail (10,20). Muscarinic receptors in brain are coupled to a guanine nucleotide binding protein (G-protein) which mediates the binding of an agonist to a receptor and the subsequent hydrolysis of phosphoinositides. There are three phosphoinositides, phosphatidylinositol (PI), phosphatidylinositol phosphate (PIP), and phosphatidylinositol bisphosphate (PIP^2). It is still not clear exactly which of these lipids is hydrolyzed following receptor activation, but current evidence suggests that both PI and PIP^2 can be hydrolyzed by receptor activation (21). Hydrolysis of PIP^2 results in the generation of two second messengers, diacylglycerol and

inositol trisphosphate (IP^3). Hydrolysis of PI produces only one second messenger, diacylglycerol. Diacylglycerol activates protein kinase C, which then phosphorylates a variety of substrate proteins. Several small reports have shown that lithium treatment alters protein phosphorylation in the brain (22-24). IP^3 stimulates the intracellular release of calcium from bound stores, resulting in an increase of the intracellular calcium concentration. There are many more complexities associated with this system that are covered in recent reviews (10,20) and cannot be adequately detailed here.

Lithium, at low concentrations (1 mM), has been shown to have a major effect on the phosphoinositide system. Sherman's group (25,26) has shown that lithium (both in vitro and in vivo) inhibits myo-inositol-1-phosphatase. Inhibition of this enzyme results in the accumulation of its substrate, IP^1, and depletion of its product, inositol, in brain, as well as in many other tissues.

Lithium administration to rats was shown to cause a dose-dependent, several-fold increase of the in vivo concentration of IP^1 (26, 27). Administration of a cholinergic agonist along with lithium resulted in a synergistic larger increase of the concentration of IP^1 (9). Treatment with atropine or scopolamine blocked the effects of lithium alone or with a cholinergic agonist (9,27), indicating that even in the absence of a cholinergic agonist, much of the in vivo phosphoinositide hydrolysis was mediated by muscarinic receptors. Recent reviews have covered in greater detail this and further evidence that has led many investigators to believe that one of the primary in vivo effects of lithium is this interaction with the phosphoinositide system (10,20,26,28,29).

In summary, it is logical to hypothesize that the interaction of lithium with the phosphoinositide system may provide the mechanism whereby lithium potentiates cholinergic agonist-induced seizures. Furthermore, it is logical to surmise that lithium is potentiating excitatory responses or attenuating inhibitory influences to reduce the seizure threshold to cholinergic agents and that these actions may also play a role in the subsequent long duration, severity, and lethality of the seizures. However, little more is presently known, especially about

how this important system is regulated. Two types of regulation have been described: modulation by protein kinase C and modulation by neurotransmitter (or neuromodulator) interactions.

Agents which stimulate phosphoinositide hydrolysis increase the production of diacylglycerol, which activates protein kinase C (10). Activation of protein kinase C by some agents, but not by others, has been shown to cause the translocation of protein kinase C from a soluble (cytoplasmic) to a particulate (membrane) fraction (30-32). Activation of protein kinase C, in turn, induces a feedback inhibition of agonist-stimulated phosphoinositide metabolism (33,34). The mechanism for this modulating inhibition is not clear; involvement of phosphorylation of receptors, G-proteins and phospholipase C has been supported (10,20). This regulatory process is perhaps the major mechanism that has been found to date for modulation of phosphoinositide metabolism. Therefore, it would be logical to examine the effects of seizures on both the soluble and particulate fractions of protein kinase C.

The second type of modulation of phosphoinositide hydrolysis that has been identified consists of interactions among neurotransmitter systems or neuromodulators. Perhaps those most widely studied are the interactions among excitatory amino acid agonists and amine neurotransmitters (35,36). For example, we and others have reported that quisqualate, a receptor subtype-selective excitatory amino acid agonist, effectively inhibits phosphoinositide hydrolysis induced by norepinephrine (NE) (37,38). The modulatory effects of the excitatory amino acids are especially relevant to the present investigation because excitatory amino acids are believed to play a major stimulatory role in many types of seizures. During the last few years a great number of reports have documented the seizure-inducing effects of excitatory amino acids and have shown that much of the neurodegeneration associated with seizures is due to activation of these pathways (35,39). As added evidence of their importance, many studies have shown that antagonists of excitatory amino acid receptors, such as MK-801 (Dizocilpine; 5-methyl-10,11-dihydro-5-dibenzo [a,d] cyclohepten-5,10-imine), are effective anticonvulsants and protect the brain from seizure-induced damage (39). It is important to note that these anticonvulsant effects

of excitatory amino acid antagonists apply to seizures induced by cholinergic agonists. For example, we recently reported (16) an extensive study of the anticonvulsant effects of MK-801 using the model of seizures induced by coadministration of lithium and pilocarpine. Pretreatment with MK-801 blocked the cholinergic agonist-induced production of generalized convulsive status epilepticus and also prevented death which otherwise always occurred with this treatment. Equally impressive was the finding that administration of MK-801 after seizures had begun resulted in termination of the seizures and blocked the death that normally occurs as a result of seizure-induced brain damage (16). These and other studies clearly demonstrate that activation of excitatory amino acid systems plays a major role in seizures, brain damage, and death associated with seizures induced by cholinergic agonists. These results emphasize the necessity of identifying the interactions of excitatory amino acids with second messenger systems, such as receptor-coupled phosphoinositide hydrolysis. As noted above, excitatory amino acids can inhibit phosphoinositide hydrolysis. It is interesting to note that in the kindling model of seizures, there is also reduced phosphoinositide hydrolysis (40). Furthermore, lithium, which potentiates cholinergic agonist-induced seizures, also reduces phosphoinositide hydrolysis (41). Thus, there is accumulating evidence that seizures are associated with inhibition of the normal activity of the phosphoinositide second messenger-generating system.

Studies of inhibitory amino acid neurotransmitters, such as GABA, provide evidence of effects opposing those of the excitatory amino acids. GABA agonists generally have anticonvulsant effects, and a preliminary report found that GABA enhanced phosphoinositide hydrolysis induced by NE (42). Thus, GABA and the excitatory amino acids appear to have opposing effects on both seizures and phosphoinositide metabolism, but very little research has been applied towards clarifying the modulatory properties of GABA on phosphoinositide hydrolysis.

The results summarized above indicate that studies of the modulation of phosphoinositide hydrolysis by excitatory and inhibitory amino acids are critical to understanding their modulatory roles in seizures. Furthermore, they indicate that use of the lithium-plus-pilocarpine model of

seizure generation is most appropriate to identify the influences of phosphoinositide metabolism on seizures and vice versa, and to understand the CNS toxicity of cholinergic agonists.

EXPERIMENTAL PROCEDURES

Animal treatments

In some experiments, rats were treated chronically with dietary LiCl. To achieve this, male Sprague-Dawley rats initially weighing approximately 130 g were fed pelleted rat chow containing LiCl (1.696 g/kg diet; Teklad-Mills, Madison, WI) or commercial rat chow for 30 days. Food, water and 0.9% saline (to prevent lithium toxicity) were provided ad libitum. This method of administration of lithium is advantageous because it produces plasma lithium concentrations (0.86 ± 0.06 mM) that are within the range considered to be therapeutic in human patients and because rats maintain body weights not significantly different from controls, unlike some lithium treatment protocols. In these studies, initial and weekly body weights were 134 ± 3, 164 ± 4, 186 ± 5, 220 ± 6, and 254 ± 5 for controls, and they were 136 ± 4, 164 ± 4, 194 ± 6, 234 ± 5 and 267 ± 7 for the lithium-treated rats ($n = 10$ for each group).

In most studies rats were treated with acute LiCl (3 mmol/kg; ip; 20 hours prior to experiments). Seizures were induced by administration of pilocarpine (30 mg/kg; sc) to lithium-treated rats. This results in paroxysmal spike activity after approximately 15 minutes, which develops into spike trains within the next 5 minutes, and finally status epilepticus develops shortly thereafter (15). Rats remain in status epilepticus for several hours until death occurs.

Brain slice preparation

Male, Sprague-Dawley rats (200-250 g) were decapitated and the brains were rapidly dissected in ice-cold 0.32 M sucrose. Slices (0.3 x 0.3 mm) from the cerebral cortex, or other regions where indicated, were prepared with a McIlwain tissue slicer and washed thoroughly with incubation media (NaCl, 122 mM; KCl, 5 mM; $MgCl_2$, 1.2 mM; KH_2PO_4, 1.2 mM; $CaCl_2$, 1.3 mM; $NaHCO_3$, 3.6 mM; glucose, 11 mM; HEPES, 30 mM; freshly bubbled with 95% O_2/5% CO_2; adjusted to pH 7.35). The slices were incubated in the same media at 37°C for 60 minutes for regeneration, followed by several washes with fresh media.

Assays of [^{3}H]inositol incorporation into slices and [^{3}H]inositol phosphate production

Two experimental protocols were used to expose slices to [^{3}H]inositol and to test the effects of different agents on [^{3}H]phosphoinositide synthesis and/or hydrolysis.

Data in Figures 1-7 and Tables 1-3 are from experiments in which slices were simultaneously exposed to [^{3}H]inositol and test agents to measure modulation of both [^{3}H]phosphoinositide synthesis and [^{3}H]inositol phosphate production as described previously (38). Aliquots of slices were incubated in a final volume of 500 µl incubation media containing 0.5 µM myo-[2-^{3}H]inositol (15 Ci/mmol), 10 mM LiCl and the indicated concentrations of test agents for 60 minutes (or other times where indicated) at 37°C. For those experiments in which proposed inhibitors of receptors, ion channels or enzymes were used, these agents were added 10 minutes before adding other agents. At the end of the incubation, samples were rapidly washed two times with 3 ml of ice-cold media and were mixed with 1.5 ml of $CHCl_3$:MeOH:12 N HCl (1:2:0.01).

For experiments shown in further figures and tables, preincubated slices were prelabelled by incubation at 37°C for 1 hour in fresh buffer containing 0.5 µM [^{3}H]inositol and 10 mM LiCl. After the incubation, the slices were rapidly washed several times to remove exogenous free [^{3}H]inositol. Aliquots of [^{3}H]inositol-prelabelled slices were incubated in 500 µl for 60 minutes or the indicated times in the presence of agonists or other agents. The reaction was stopped by adding 1.7 ml of $CHCl_3$:MeOH:12 N HCl (1:2:0.01).

Samples obtained from either assay were transferred to extraction tubes and mixed with 1 ml of $CHCl_3$ and 0.5 ml H_2O. The lipid phase was separated from the aqueous phase by centrifugation. Aqueous fractions were mixed with 0.5 ml of a 50% slurry of AG1-X8 resin, [^{3}H]inositol phosphates were separated by the method of Berridge et al. (43), as described previously (34), and the radioactivity in each sample was measured. The lipid phase was dried overnight at room temperature and the radioactivity was measured. Data were analyzed using 2-way analysis of variance (ANOVA) or Student's t-test.

Membrane [^{3}H]PI hydrolysis

The methods used for preparation of membranes and measurement of [^{3}H]PI hydrolysis were exactly as described by Wallace and Claro (44). Final incubation mixtures (0.1 ml) contained 100 µg membrane protein, 10 mM tris-maleate (pH 6.8), 6 mM $MgCl_2$, 8 mM LiCl (except where noted otherwise), 3 mM EGTA (ethylene glycol-bis (β-aminoethyl ether) N,N,N^1,N^1-tetracetic acid) and sufficient $CaCl_2$ to yield a free Ca^{2+} concentration of 0.3 µM, 1 mM deoxycholate, 0.1 mM [^{3}H]PI (5-10 x 10^4 cpm; New England Nuclear, Boston, MA), and other additions where indicated. $AlCl_3$ (10 µM) was always included in incubations containing NaF to allow formation of the active species, AlF. Reactions continued for 20 minutes at 37°C and were stopped by addition of 1.2 ml $CHCl_3$:CH_3OH (1:2). $CHCl_3$ (0.5 ml) and 0.25 N HCl (0.5 ml) were added, samples were mixed, placed on ice for 10 minutes, phases were separated by centrifugation, and an aliquot of the aqueous phase was counted for radioactivity. Each assay was carried out in triplicate on a membrane preparation from a brain region from an individual rat, and ANOVA was used to determine statistical significance. Data from all control and treated rats in the groups used for each figure were pooled and analyzed as a group (i.e., no normalization or pairing procedures were necessary).

In vivo Inositol 1,4,5 trisphosphate (Ins 1,4,5 P_3)

All rats were killed by a beam of microwave irradiation focussed on the head (Gerling Labs, Modesto, CA) to prevent postmortem changes in the concentrations of the second messengers. The concentration of Ins 1,4,5P_3 was measured by a radioreceptor binding assay (45) available commercially (Amersham, Arlington Heights, IL) using the protocol exactly as described by Whitworth et al. (46).

Preparation of subcellular fractions

Each rat was decapitated, and the brain was rapidly removed and placed in ice-cold 0.32 M sucrose. The hippocampus was isolated and homogenized in 10 volumes of 10 mM tris-Cl, pH 7.4, containing 2 mM EDTA (ethylenediaminetetraacetric acid), 0.5 mM EGTA, 20 µg/ml leupeptin, 10 mM benzamidine, and 0.1 mM phenylmethylsulfonyl fluoride (buffer A). The

homogenate was centrifuged at 100,000 x g for 1 hour at 4°C. After collection of the supernatant (soluble fraction) on ice, the pellet (particulate fraction) was resuspended in an equivalent volume of buffer A containing 0.2% Nonidet P-40 (NP-40) and stirred for 30 minutes at 4°C (47). Protein concentration was determined (48) using bovine serum albumin (BSA) as the standard. Protein concentrations of each fraction were adjusted to 0.1 mg/ml for the protein kinase C assay and 2 mg/ml for the assay of endogenous protein phosphorylation.

Assay of protein kinase C activity

Protein kinase C activity was measured at 30°C using assay procedures adapted from Kikkawa et al. (47), Castagna et al. (49), and Zatz (50). Phosphorylation was performed in a reaction mixture (0.2 ml, final volume) which contained 25 mM tris-Cl, pH 7.4, 6 mM $MgSO_4$, 1 mM EDTA, 1 mM EGTA, 1 mM ß-mercaptoethanol, 1.5 mM $CaCl_2$, 50 µg of histone (Type IIIS, Sigma Chemical Co., St. Louis, MO), 2 µg sample protein, and 30 µM [γ-^{32}P]ATP (40 Ci/mol) in the presence or absence of 10 µg phosphatidylserine (PS) and 1 µM phorbol 12-myristate 13-acetate (PMA). The reaction mixture without [γ-^{32}P]ATP was preincubated for 30 seconds, followed by addition of [γ-^{32}P]ATP to initiate phosphorylation. After 2 minutes, phosphorylation was stopped by addition of 0.5 ml ice-cold 10% trichloroacetic acid (TCA) containing 5 mM ATP and 20 mM NaH_2PO_4. BSA (100 µg) was added and the precipitated protein was collected onto Whatman GF/B membrane filters. The filters were washed three times with 5 ml 5% TCA, dried, and radioactivity was measured by liquid scintillation spectrometry. Protein kinase C activity was expressed as nanomole ^{32}P incorporated/min/mg protein and calculated as the difference between phosphorylation measured in the presence or absence of PS and PMA.

Phosphorylation of endogenous proteins and sodium dodecyl sulfate (SDS) gel electrophoresis

Phosphorylation of endogenous proteins was conducted at 30°C in a reaction mixture (0.2 ml, final volume) containing 25 mM tris-Cl, pH 7.4, 6 mM $MgSO_4$, 1 mM EDTA, 1 mM EGTA, 1 mM ß-mercaptoethanol, and 100 µg sample protein in the presence of either (a) no further additions, (b) 1.5 mM $CaCl_2$, (c) 1.5 mM $CaCl_2$,10 µg PS, and 1 µM PMA, (d) 1 mM

theophylline, or (e) 1 mM theophylline and 50 μM cAMP. After preincubation for 30 seconds, phosphorylation was initiated by the addition of 2 μM [γ-^{32}P]ATP (2500 Ci/mol). Phosphorylation was stopped after 30 seconds by addition of 100 μl of SDS stop solution containing (final concentrations) 93.8 mM tris-Cl, pH 6.8, 25% glycerol, 3% SDS, 10% ß-mercaptoethanol, with bromphenol blue as the dye front marker, and each sample was placed in a boiling water bath for 2 minutes.

A portion of each sample (containing 20-30 μg protein) was subjected to one-dimensional SDS-polyacrylamide (6.5% or 12%) gel electrophoresis (SDS-PAGE) as described by Laemmli (51). The gels were stained for 2 hours with Coomassie Brilliant Blue R-250, destained overnight, and dried. Dried gels were exposed to Kodak X-OMAT AR film, and the autoradiographs were scanned with a GS 300 transmittance/reflectance scanning densitometer (Hoeffer Scientific Instruments, San Francisco, CA). Protein kinase C-mediated phosphorylation was measured by subtracting peak heights obtained with calcium alone from those in the presence of calcium, PS, and PMA. Cyclic AMP-dependent protein phosphorylation was measured by subtracting peak heights obtained with theophylline alone from those in the presence of theophylline and cAMP. These data were expressed as percent of control values (peak height of lithium-treated/peak height of control X 100). Apparent molecular masses of the phosphoproteins in kilodaltons (kD) were determined from protein standards: lysozyme (14.4 kD), soybean trypsin inhibitor (21.5 kD), carbonic anhydrase (31 kD), ovalbumin (45 kD), BSA (66.2 kD), phosphorylase B (92.5 kD), ß-galactosidase (116 kD), and myosin (200 kD) (BioRad; Richmond, CA).

Protein tyrosine phosphorylation

The brain regions indicated were rapidly removed, weighed, and homogenized in 10 volumes of 125 mM tris (pH 6.8), 2% SDS, 10% glycerol, 5% ß-mercaptoethanol and 1 mM sodium vanadate. The homogenates were sonicated for 30 seconds and placed in a boiling water bath for 5 minutes. The protein concentration was measured (52) after acid precipitation of the protein. Samples were diluted with Laemmli sample buffer and 80 μg protein, and were run on

7.5% SDS-polyacrylamide gels, transferred to nitrocellulose (53) and probed with a monoclonal antibody to phosphotyrosines (1:1000, PY 20; ICN Immunobiologicals, Lisle, IL). After incubation with the primary antibody, the blots were incubated with horseradish peroxidase labelled goat-antimouse IgG and color developed with 3,3'-diaminobenzidine in the presence of hydrogen peroxide. The immunoblots were dried and the bands were quantitated using a BioRad Video Densitometer 620. Band areas of samples from control and treated rats were compared and the results from several preliminary experiments were used to choose proteins for quantitative analysis, including those in which possible changes occurred and some which appeared not to be affected by the treatments under study. Statistically significant differences were determined by ANOVA.

RESULTS

Our initial experiments established optimal conditions for quantifying the activity of the phosphoinositide second messenger generating system in slices from regions of rat brain. We characterized the concentration-dependence for carbachol and NE to induce phosphoinositide hydrolysis, and in preliminary studies showed that glutamate inhibited agonist-induced phosphoinositide hydrolysis. This inhibitory effect of glutamate was found to be due to activation of the quisqualate-selective receptor. Therefore, the initial portion of the present investigation was undertaken to examine in more detail the modulation of phosphoinositide hydrolysis by excitatory amino acids.

Effects of calcium concentration on [^{3}H]phosphoinositide synthesis and hydrolysis and the inhibitory effect of glutamate

Figure 1 shows that calcium had two opposing effects on the phosphoinositide system in cortical slices. Increasing the calcium concentration reduced the incorporation of [^{3}H]inositol into [^{3}H]phosphoinositides (Figure 1A), as reported previously (34). This inhibitory effect occurred irrespective of whether or not NE or carbachol was included in the incubation medium. Glutamate significantly inhibited the synthesis of [^{3}H]phosphoinositides in all conditions except

in the presence of EGTA, and the percent inhibition by glutamate increased as the calcium concentration was increased (e.g., glutamate in the presence of NE inhibited [^{3}H]phosphoinositide synthesis by 16 ± 4, 27 ± 2, 33 ± 2, and 37 ± 2% in EGTA, no added calcium, 0.1 mM and 1.3 mM calcium, respectively).

In contrast to [^{3}H]phosphoinositide synthesis, agonist-stimulated [^{3}H]IP^1 production required calcium and was severely reduced as the concentration of calcium was decreased (Figure 1B). Glutamate significantly inhibited NE-stimulated [^{3}H]IP^1 production under all conditions except in the presence of EGTA, but had no significant effect on carbachol-stimulated [^{3}H]IP_1 production. As with [^{3}H]phosphoinositide synthesis, the percent inhibition of NE-stimulated [^{3}H]IP_1 production caused by glutamate increased as the calcium concentration was increased, resulting in 6 ± 1, 31 ± 2, 37 ± 5 and 49 ± 7% inhibition in the four conditions.

A calcium channel blocker, verapamil, in media containing 1.3 mM calcium, resulted in a concentration-dependent increase of the synthesis of [^{3}H]phosphoinositides in the absence or presence of NE (Figure 2A) which is consistent with the finding that reduced calcium enhances [^{3}H]phosphoinositide synthesis. Glutamate significantly ($p < 0.05$) reduced [^{3}H]phosphoinositide synthesis in the presence of NE at all concentrations of verapamil.

Verapamil caused a significant inhibition of NE-induced [^{3}H]IP_1 production (Figure 2B), in contrast to its stimulation of [^{3}H]phosphoinositide synthesis. NE-stimulated [^{3}H]IP_1 production was significantly reduced by glutamate in the absence of verapamil or with 10, 30 and 50 μM verapamil. However, with 75 or 100 μM verapamil there was not a statistically significant inhibition of glutamate, as the inhibitory effect of verapamil had reduced the response to NE to near that observed in the presence of NE and glutamate.

Effects of quisqualate on [^{3}H]phosphoinositide synthesis and hydrolysis

Previous observations indicated that the inhibitory effect of glutamate on NE-stimulated [^{3}H]IP_1 production was mimicked by quisqualate, but not by kainate or N-methyl-D-aspartate (NMDA) (38). Quisqualate produced a concentration-dependent inhibition of [^{3}H]phosphoinositide synthesis (Figure 3A). As reported by others (54), 100 μM quisqualate

caused a small but statistically significant stimulation of [^{3}H]IP$_1$ formation, both in the presence and absence of NE, whereas higher concentrations of quisqualate inhibited this process (Figure 3B). This biphasic effect of quisqualate makes it difficult to obtain a clear measurement of the inhibition of NE-stimulated [^{3}H]IP$_1$ production, as both quisqualate-mediated responses may be activated at concentrations near 10^{-4} M. The biphasic response to quisqualate also necessitates the use of relatively high concentrations to study the inhibitory portion of its action.

None of several excitatory amino acid receptor antagonists blocked quisqualate-mediated inhibition of [^{3}H]phosphoinositide synthesis or of the response to NE (Figure 4), including the relatively specific quisqualate receptor-selective antagonist CNQX. AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolpropionate), which is an agonist apparently selective for a subpopulation of quisqualate receptors (35), did not alter [^{3}H]phosphoinositide synthesis or NE-induced [^{3}H]IP$_1$ production. These observations support previous suggestions that the inhibitory effect of quisqualate is mediated by a unique receptor that is insensitive to the known excitatory amino acid receptor antagonists (36) and is not activated by AMPA.

Effects of arachidonic acid on [^{3}H]phosphoinositide synthesis and hydrolysis

Excitatory amino acids and calcium can stimulate phospholipase A$_2$ (55), resulting in the liberation of arachidonic acid. Therefore, we tested the possibility that arachidonic acid may modulate [^{3}H]phosphoinositide synthesis and its hydrolysis stimulated by NE. Concentrations of arachidonic acid of 50 μM and greater significantly reduced the synthesis of [^{3}H]phosphoinositides (Figure 5A) and NE-stimulated [^{3}H]IP$_1$ production (Figure 5B). Both effects of arachidonic acid were prevented by inclusion of BSA (1 mg/ml), which binds free fatty acids in the incubation medium (data not shown). Thus, as found with glutamate and quisqualate, arachidonic acid caused concomitant inhibition of both [^{3}H]phosphoinositide synthesis and the hydrolysis stimulated by NE.

The time course of these inhibitory effects of arachidonic acid (Figure 6) was similar to those of glutamate and quisqualate, as reported previously (38). [^{3}H]Phosphoinositide synthesis was inhibited by arachidonic acid within 10 minutes and synthesis remained depressed through

60 minutes of incubation. NE did not significantly stimulate $[^3H]IP_1$ production until 40 minutes, as reported previously, due to the need to significantly label the pool of lipids available to the receptor (38), and arachidonic acid significantly depressed this response to NE. Arachidonic acid produced similar inhibitory effects on NE-stimulated production of $[^3H]IP_2$ and $[^3H]IP_3$ (data not shown).

In the presence of carbachol, arachidonic acid also inhibited the synthesis of $[^3H]$phosphoinositides and the stimulated production of $[^3H]IP_1$ (Figure 7). This indicates that the inhibitory effect of arachidonic acid is not specific for only adrenergic receptor-containing cells.

Calculation of the ratio $[^3H]IP_1/[^3H]IP_1$ + $[^3H]$phosphoinositides provides an indication of the activity of receptors coupled to phosphoinositide hydrolysis. This ratio was unchanged by arachidonic acid compared with either NE or carbachol alone (Table 1), suggesting that the inhibitory effect of arachidonic acid is nonselective with regards to pools of phosphoinositides and that its inhibitory effect on $[^3H]IP_1$ hydrolysis is likely to be due to the impaired synthesis of $[^3H]$phosphoinositides rather than to impaired function of the receptor complex.

To examine if $[^3H]$phosphoinositide synthesis and the production of $[^3H]IP_1$ were inhibited directly by arachidonic acid or by products of its metabolism, we tested several inhibitors of arachidonic acid metabolism, including indomethacin (cyclooxygenase inhibitor), nordihydroguaiaretic acid (NDGA, lipoxygenase inhibitor) and phenidone (inhibitor of both cyclooxygenase and lipoxygenase), each at a final concentration of 100 μM. Indomethacin and phenidone, but not NDGA, had weak inhibitory effects of their own, and none of these agents reduced the inhibition caused by 200 μM arachidonic acid (n=2; data not shown). This indicates that arachidonic acid itself may cause the inhibition of $[^3H]$phosphoinositide synthesis and hydrolysis.

Agents known to modulate the activity of phospholipase A_2 were tested for direct effects on $[^3H]$phosphoinositide synthesis and NE-stimulated $[^3H]IP_1$ formation, and for modulatory effects on the inhibition of these processes induced by quisqualate. Table 2 shows that activation

of phospholipase A_2 activity by melittin caused large reductions of [^{3}H]phosphoinositide synthesis and [^{3}H]IP_1 production. This was apparently a widespread effect, not localized to certain cells, since the calculated ratio did not change, similar to the response to arachidonic acid. Inhibitors of phospholipase A_2 including chloroquine, bromphenacyl bromide (BPB), and dexamethasone were tested in this system although none of these agents is highly specific for phospholipase A_2. Each of these inhibitors produced some inhibition of [^{3}H]phosphoinositide synthesis, and chloroquine and BPB significantly reduced NE-stimulated [^{3}H]IP_1 production. None of these agents blocked the inhibitory effects of quisqualate.

Experiments were carried out to determine if any of several agents altered the incorporation of [^{3}H]inositol into phosphoinositides mediated by the Mn^{2+}-activated base exchange reaction. Mn^{2+} increased the incorporation of [^{3}H]inositol into phosphoinositides (56) and NE did not alter the rate of the base exchange reaction (Table 3). Glutamate, quisqualate, and arachidonic acid each inhibited [^{3}H]phosphoinositide synthesis in the absence of Mn^{2+}. Only arachidonic acid reduced the base exchange-mediated incorporation of [^{3}H]inositol into phosphoinositides, but the percent inhibition was much less than that obtained in the absence of Mn^{2+}.

Effects of amino acids on agonist-stimulated [^{3}H]phosphoinositide hydrolysis in prelabelled slices

The previous experiments utilized a method in which slices were exposed simultaneously to [^{3}H]inositol and test agents to allow measurements of both synthesis and hydrolysis of [^{3}H]phosphoinositides. In the following experiments, slices were prelabelled with [^{3}H]inositol and subsequently thoroughly washed before agonists and other drugs were added in an attempt to dissociate modulatory effects on [^{3}H]IP_1 production from those on [^{3}H]phosphoinositide synthesis.

Because there is greater synthesis of [^{3}H]phosphoinositides in the absence than in the presence of added calcium (34), each condition was used (without added EGTA) during the prelabelling incubation prior to the measurement of [^{3}H]IP_1 production (which was always

carried out in media containing 1.3 mM calcium). These experiments revealed further complexities in the role of calcium in phosphoinositide hydrolysis. Although the synthesis of [^{3}H]phosphoinositides was higher in the absence of calcium (as shown in Figure 1), the subsequent response to NE was depressed (Figure 8). Additionally, the modulatory effects of the amino acids on NE-stimulated [^{3}H]IP_1 production were blunted in slices prelabelled without calcium, suggesting that reduced calcium attenuates the inhibitory effects of the modulatory amino acids. However, in the slices incubated continuously in the presence of calcium there were clear modulatory influences by the amino acids. [^{3}H]IP_1 production induced by NE was inhibited by both glutamate and quisqualate, unaltered by kainate, surprisingly enhanced by NMDA, and inhibited by homocysteic acid and L-cysteine. Thus, this condition was used in further studies reported below.

Examination of the NE concentration-dependent stimulation of [^{3}H]IP_1 production revealed that quisqualate significantly reduced the maximal response to NE (Figure 9).

The inhibitory effects of excitatory amino acids on both phosphoinositide synthesis and NE-stimulated inositol phosphate production raised the question of the size of the pool of phosphoinositides available to the α1-adrenergic receptor and its rate of turnover in the presence of NE. That is, in prelabelled slices, is it possible to inhibit NE-stimulated [^{3}H]IP_1 production by inhibition of further synthesis of [^{3}H]phosphoinositides? To examine this, following prelabelling of slices with [^{3}H]inositol and washing away extracellular [^{3}H]inositol, unlabelled inositol (1,5, or 20 mM final concentration) was equilibrated with slices for 15 minutes followed by incubation with NE for 15 or 30 minutes. Figure 10 shows that there was an inositol-induced concentration-dependent and time-dependent reduction of NE-stimulated [^{3}H]IP_1 production. The results with 20 mM inositol may be the most relevant because high concentrations have been reported to be required to dilute endogenous [^{3}H]inositol (57). With 20 mM inositol, during the first 15 minutes of incubation with NE, the production of [^{3}H]IP_1 was reduced by 53%, and by 30 minutes it was reduced by over 75%. These results suggest that phosphoinositides available to the α1-adrenergic receptor complex turn over to a significant extent during these incubation

periods and that inhibition of [^{3}H]phosphoinositide synthesis may contribute to inhibition of [^{3}H]IP_1 production even in prelabelled slices. Alternatively, however, it is possible that high concentrations of inositol are capable of directly displacing [^{3}H]inositol from labelled phosphoinositides through homologous base exchange reactions.

Excitatory amino acids have been reported to induce chloride influx (58) as well as polyamine synthesis (59), so the effects of antagonists of these processes were measured on the response to NE and its inhibition caused by quisqualate. Neither furosemide nor diisothiocyanotostilbene-2,2-sulfonic acid (DIDS) blocked the inhibitory effect of quisqualate, although each of these agents slightly increased the response to NE (Table 4). Similarly, α-difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase, did not block the inhibition induced by quisqualate.

Modulation by GABA of agonist-stimulated [^{3}H]phosphoinositide hydrolysis in prelabelled slices

Because GABA has been reported to enhance [^{3}H]IP_1 production in response to NE (42), the effects of GABA on stimulation by agonists and inhibition by excitatory amino acids and arachidonic acid were examined. GABA produced a biphasic concentration-dependent modulation of NE-stimulated [^{3}H]IP_1 production in cortical slices (Figure 11). The response to a maximal concentration of NE (200 μM) was enhanced by 30 and 100 μM GABA and was inhibited by 1 and 10 mM GABA. There was a greater enhancement of the response to a submaximal concentration of NE (2 μM). GABA did not alter carbachol-stimulated [^{3}H]IP_1 production, nor did it alter inhibition by glutamate of [^{3}H]IP_1 produced in response to 200 μM NE.

The effects of GABA were found to be regional-specific. In agreement with previous reports (42), GABA alone caused no stimulation of [^{3}H]IP_1 production in any of the brain regions that were tested. In striatal slices, GABA enhanced the response to carbachol, but did not affect that of NE or ibotenate (Figure 12). In hippocampal slices, GABA enhanced the response to carbachol and to ibotenate, although different concentrations of GABA were effective with

each of these agonists (Figure 13). GABA also enhanced the response to NE, and this increase in hippocampal slices was greater than that observed in cortical slices. The maximal effect of GABA was obtained at a concentration of 300 μM which increased the response to NE throughout the range of effective concentrations of NE, producing from 69% to 30% increases (Figure 14).

The effects on NE-stimulated [^{3}H]IP_1 production of combinations of GABA and inhibitory agents were tested. Quisqualate and arachidonic acid significantly inhibited NE-induced [^{3}H]IP_1 production in slices that had been prelabelled with [^{3}H]inositol and baclofen, a specific $GABA_B$ agonist, was as effective as GABA in enhancing the response to NE (Figure 15). Neither GABA nor baclofen significantly blocked the inhibitory effect of quisqualate, but baclofen reduced the inhibitory effect of arachidonic acid.

Effects of NMDA receptor antagonists on phosphoinositide hydrolysis

MK-801 is an NMDA receptor antagonist which is an effective anticonvulsant in many seizure models, including seizures induced by administration of lithium and pilocarpine (16). In experiments using MK-801 to block the modulatory effects of excitatory amino acids on phosphoinositide hydrolysis, we noted that MK-801 itself was able to activate phosphoinositide metabolism. Therefore, this response to MK-801 was investigated in further detail.

MK-801 induced a concentration-dependent (EC_{50} ~ 380 μM) increase of phosphoinositide hydrolysis (Figure 16). The maximum effect occurred at 1 mM MK-801 and was 4.5-fold of basal [^{3}H]-IP_1 accumulation. AP-7, another NMDA antagonist, did not affect phosphoinositide turnover, and PCP (phencyclidine) did not induce phosphoinositide hydrolysis until 1 mM. Phosphoinositide hydrolysis stimulated by 500 μM MK-801 was not significantly affected by NMDA (1 mM) or glycine (0.1, 1.0 mM) alone, or in combination, or by Zn^{2+} (0.5 mM) (Figure 17). Removal of Mg^{2+} from the assay medium did not significantly affect the MK-801-induced phosphoinositide hydrolysis (Figure 17). The MK-801-induced response in the absence of Mg^{2+} was not significantly affected by NMDA (1 mM) or glycine (0.1, 1.0 mM),

alone or in combination. The MK-801 response was unaffected by either prazosin (10 μM) or atropine (10 μM) (data not shown).

Modulation of phosphoinositide hydrolysis by Na^+

Excitatory amino acids function in part by activating ion channels, which can result in the influx of Na^+ (35). To examine if Na^+ influx was necessary for the modulatory effects of excitatory amino acids on phosphoinositide hydrolysis, the concentration of Na^+ in the extracellular incubation medium was reduced. This was observed to have a number of important effects on phosphoinositide hydrolysis.

The accumulation of $[^3H]IP_1$ was measured after a 60 minute incubation of [^{3}H]inositol-prelabelled cerebral cortical slices in media containing variable concentrations of Na^+ (Figure 18). In the absence of added agonist (basal), reduction of the Na^+ concentration from 120 mM Na^+ in controls resulted in enhanced accumulation of $[^3H]IP_1$, with maximal increases of approximately sevenfold occurring in media with 0 or 5 mM Na^+. NE (100 μM) significantly stimulated the production of $[^3H]IP_1$ in 120 mM Na^+, and this response was increased incrementally as the Na^+ concentration was reduced. The inset in Figure 18 shows the difference in the $[^3H]IP_1$ produced in media with reduced Na^+ compared with that in 120 mM Na^+, demonstrating that the enhanced response to NE is greater than and independent of the increase in the basal rate of $[^3H]IP_1$ production. Prazosin (10 μM), an α-adrenergic receptor antagonist, completely blocked the response to NE in 120 and 5 mM Na^+ (data not shown).

Incubation with the sodium channel blocker tetrodotoxin (TTX) did not cause the same effects as did removal of Na^+ from the medium, indicating that Na^+ influx through voltage-dependent channels is not responsible for the depressed phosphoinositide hydrolysis observed in 120 mM Na^+. Basal and NE-stimulated $[^3H]IP_1$ production were unaffected by inclusion of TTX in the medium, while the response to carbachol was significantly reduced by TTX concentrations of 0.3 μM and greater (Figure 19).

Figure 20 shows the agonist-selectivity of the influence of Na^+ on phosphoinositide hydrolysis. Phosphoinositide hydrolysis stimulated by NE was enhanced in low Na^+, and this

increase was more than additive with the increased [^{3}H]IP_1 produced under basal conditions. In contrast, the responses to carbachol and ibotenate were only slightly enhanced in low Na^+, and these increases appeared to be additive with the larger basal response, indicating that phosphoinositide hydrolysis induced by these two agents was not sensitive to the Na^+ concentration. The most impressive Na^+-sensitivity was observed with quisqualate which produced a large increase in [^{3}H]IP_1 accumulation in media with low Na^+.

We recently reported that glutamate, arachidonate, and quisqualate significantly inhibited NE-stimulated phosphoinositide hydrolysis in rat cerebral cortical slices (60). Figure 20 shows that glutamate (0.5 mM) reduced [^{3}H]IP_1 produced in response to NE by approximately 50% and that this degree of inhibition was not altered in media with low Na^+. Arachidonate (0.2 mM) inhibited the response to NE by approximately 40% in 120 mM Na^+ and this inhibition was only slightly reduced (to approximately 25%) in 5 mM Na^+. In contrast, whereas quisqualate (0.5 mM) inhibited the response to NE by 60% in 120 mM Na^+, there was no inhibition by quisqualate evident in 5 mM Na^+. Tetrodotoxin (TTX) did not affect the inhibitory effects of glutamate, arachidonate, or quisqualate.

To examine if the apparent absence of an inhibitory effect of quisqualate in low Na^+ was actually due to its being masked by enhanced quisqualate-stimulated phosphoinositide hydrolysis, we employed another excitatory amino acid agonist, L-BOAA (β-N-oxalyl-L-α, β-diaminopropionate). As reported previously (61), L-BOAA mimics the inhibition caused by quisqualate but does not itself induce [^{3}H]IP_1 production in 120 mMNa^+ (Figure 21). In 5 mM Na^+, inhibition by L-BOAA of the response to NE was totally blocked, while L-BOAA (1 mM) alone produced only a small rise in [^{3}H]IP_1, which was much below that caused by quisqualate (Figure 20). These results reinforce the conclusion that L-BOAA is more selective than is quisqualate in causing inhibition of NE-stimulated phosphoinositide hydrolysis without activating this process. Furthermore, the data indicate that the two responses to quisqualate are differentially affected by low Na^+, supporting the hypothesis that they are mediated by different receptors or mechanisms.

The enhancement by low Na^+ of quisqualate-stimulated $[^3H]IP_1$ production was examined further by measuring the concentration-dependence of this response. In the presence of 5 mM Na^+ there was a remarkable enhancement of the potency of quisqualate so that $10^{-7}M$ quisqualate significantly elevated $[^3H]IP_1$ (Figure 22). This is about a 100-fold lower concentration than is required to activate phosphoinositide hydrolysis in 120 mM Na^+. Also, the maximal response to quisqualate was almost sixfold higher in 5 mM Na^+ than in 120 mM Na^+. These large effects of Na^+ on quisqualate response contrast with the lack of Na^+-sensitivity of ibotenate-stimulated phosphoinositide hydrolysis. Additionally, the inhibition caused by the higher concentrations of quisqualate in 120 mM Na^+ are greatly attenuated in 5 mM Na^+.

Phosphoinositide hydrolysis in hippocampal slices was also influenced by the Na^+ concentration. Figure 23 shows that compared with 120 mM Na^+, in 5 mM Na^+ $[^3H]IP_1$ production was increased under basal conditions and in response to NE and quisqualate. Also shown in Figure 23 are the findings that in 5 mM compared with 120 mM Na^+, there were much greater accumulations of $[^3H]IP_2$ and slightly increased accumulations of $[^3H]IP_3$ in response to quisqualate or NE.

Protein kinase C activity

Stimulation of phosphoinositide hydrolysis results in the production of diacylglycerol which activates protein kinase C. Therefore, we established methods to study protein kinase C, including measurements of the enzyme activity and of the phosphorylation of proteins present in particulate (membrane) or soluble (cytosolic) preparations from rat brain regions. Initial experiments were carried out to make these measurements in brain samples from control and lithium-treated rats in preparation for studying changes following induction of seizures by administration of lithium and cholinergic agonists.

The activity of protein kinase C in the soluble and particulate fractions from hippocampus of control and lithium-treated rats is summarized in Table 5. The relative distribution of protein kinase C activity in control soluble and particulate fractions was 39% and 61%, respectively. This is in agreement with previously published data (47). As a prelude to measuring the effects

of seizures induced by lithium plus pilocarpine on protein kinase C activity, the effects of lithium were first tested using chronic lithium treatment rather than acute lithium treatment to obtain stable brain lithium concentrations. Chronic lithium treatment did not alter the relative distribution of this enzyme activity. Also, there were no statistically significant differences (Student's t-test, $p > 0.05$) in specific activity or total protein kinase C activity in the hippocampal particulate or soluble fractions prepared from chronic-lithium treated rats when compared with control values.

Endogenous protein phosphorylation

Particulate and soluble fractions prepared from hippocampus of control and chronic lithium-treated rats were incubated with either calcium alone and calcium with PS and the phorbol ester PMA (to activate protein kinase C), or theophylline alone and theophylline with cAMP. This allowed measurement of phosphorylation of endogenous proteins by protein kinase C and cAMP-dependent protein kinase, respectively. Basal phosphorylation was measured in the absence of any of these additions. Electrophoresis was performed on 6.5% or 12% polyacrylamide gels to separate phosphoproteins of apparent molecular mass greater than 45 kD or less than 45 kD, respectively.

Particulate fraction: Endogenous and stimulated protein phosphorylation

In the particulate fraction, under basal conditions, a major band of phosphoprotein was observed at an apparent molecular mass of 47 kD; intermediate bands were observed at 50, 115, and 138 kD, and several minor bands appeared (Figure 24). There were no significant differences in the phosphorylation of these proteins between control and chronic lithium-treated rats (data not shown).

With the addition of calcium, phosphorylation of at least three proteins of apparent molecular masses 45, 58, and 72 kD was increased compared with basal conditions (Fig. 24). After chronic lithium treatment, phosphorylation of these proteins was slightly reduced (to 90 ± 17%, 84 ± 7%, and 88 ± 7%, of controls, respectively) but the differences were not statistically significant.

Activation of protein kinase C with PS and PMA increased the phosphorylation of a number of proteins in the particulate fraction (Figure 24). The phosphorylation of several high-molecular-mass proteins of 45, 76, 87, 158, and 200 kD (Figure 24), and three low-molecular-mass proteins of 16, 18, and 19 kD (Figure 24) was most clearly increased. Phosphorylation of most of the protein kinase C substrate proteins was unaltered by chronic lithium treatment, but there were statistically significant decreases in the phosphorylation of the 18, 19, and 87 kD proteins (Table 6).

In the presence of the theophylline alone (Figure 25), no significant change in particulate protein phosphorylation was observed compared with basal conditions. Incubation with theophylline and cAMP stimulated the phosphorylation of several proteins in the particulate fraction (Figure 25). Chronic lithium treatment significantly reduced the phosphorylation of the 54 and 71 kD proteins (Table 6).

Soluble fraction: Endogenous and stimulated protein phosphorylation

Upon incubation of the soluble fraction under basal conditions, phosphorylation was limited to a few faint bands (data not shown). No significant differences in the phosphorylation of soluble proteins between the control and lithium-treated samples were observed in these samples.

Incubation with calcium increased the phosphorylation of proteins with apparent molecular masses of 46 and 56 kD (Figure 26). As in the particulate fraction, incubation of soluble fractions with calcium had little effect on the phosphorylation of lower molecular mass proteins (Figure 3B). In samples from chronic lithium-treated rats, no significant alterations in phosphorylation were observed in comparison with controls.

Activation of protein kinase C led to increased phosphorylation of a large number of proteins in the soluble fraction (Figure 26). In samples from chronic lithium-treated rats, phosphorylation was increased significantly in four proteins of apparent molecular masses of 16, 17, 20 and 22 kD (Table 7). The phosphorylation of several other proteins also appeared to be

increased, but, due to the large variations in the percent stimulation of phosphorylation after chronic lithium treatment compared with controls, the increases were not statistically significant.

Incubation of the soluble fraction with cAMP primarily increased phosphorylation of a 260 kD protein band, and to a lesser extent, the 48, 49, 69, 70, and 126 kD phosphoproteins. Chronic lithium treatment did not significantly alter the phosphorylation of these proteins compared with controls (data not shown).

Effects of seizures on phosphoinositide hydrolysis

Following the identification of modulators of phosphoinositide hydrolysis, we investigated if seizures induced in rats by administration of lithium and pilocarpine affected phosphoinositide metabolism.

Phosphoinositide hydrolysis was measured in hippocampal slices from control, lithium-treated rats, or lithium plus pilocarpine-treated rats, the latter being either 25 minutes post-pilocarpine, near the initiation of status epilepticus, or after 90 minutes, when status epilepticus had continued for just over one hour. Treatment with only lithium or pilocarpine (data not shown) did not alter the response to any of the agonists that were tested. However, seizures induced by lithium plus pilocarpine caused decreased responses to ibotenate and to NE (Figure 27). There were significant decreases with these two agonists in the samples from rats where seizures had just initiated (25 minutes) as well as further decrements after an hour of continuous seizures (90 minutes). In contrast to these effects of seizures, there were no changes in phosphoinositide hydrolysis induced by carbachol, carbachol plus 15 mM K^+ (which potentiates the response to carbachol), or 55 mM K^+, in any of the treatment groups.

The reduced response to NE in hippocampal slices after seizures was examined in greater detail by measuring the concentration-dependence of NE-induced phosphoinositide hydrolysis (Figure 28). These results demonstrated that after seizures there were decreased responses to NE at concentrations of 3×10^{-6}M and greater, and that the major effect of seizures was the reduction of the maximal response to NE. It is also evident that the largest deficit occurred

rapidly after the initiation of seizures (25 minutes) and that further decreases (90 minutes after pilocarpine) were relatively smaller.

Similar neurotransmitter-specific impairments of phosphoinositide hydrolysis were observed in cortical slices from rats undergoing seizures induced by lithium plus pilocarpine. Figure 29 shows that phosphoinositide hydrolysis induced by carbachol was similar in cortical slices from control and seizing rats, while the response to NE was reduced by seizures. Quisqualate, another excitatory amino acid agonist, caused less stimulation of phosphoinositide hydrolysis in cortical slices from seizing rats than from controls. Quisqualate also inhibits NE-induced phosphoinositide hydrolysis, and this inhibitory influence was not altered by seizures, as 0.5 mM quisqualate reduced the response to NE by approximately 50% in control and treated samples.

As detailed earlier in this report phosphoinositide hydrolysis stimulated by NE and quisqualate (but not carbachol) is enhanced when the concentration of Na^+ in the incubation medium is reduced to 5 mM. The results in Figure 29 demonstrate these effects of low Na^+ and show that in slices from seizing rats, the responses to NE and quisqualate remain below controls, even when measured in 5 mM Na^+. Lowered Na^+ greatly reduces the inhibitory influence of quisqualate on NE-stimulated phosphoinositide hydrolysis, and this effect was evident in slices from control and seizing rats. Thus, the stimulatory response to quisqualate as well as to NE was reduced by seizures, but the inhibitory effect of quisqualate on NE-induced phosphoinositide hydrolysis was not altered by seizures.

Since the responses to ibotenate and NE were altered by seizures, we examined whether the effects of modulators of these two agonists were also altered. Table 8 shows that, as reported previously quisqualate greatly inhibited the response to NE. This inhibitory effect of quisqualate was unaltered by lithium or seizures. Table 8 also shows that AP-4, an antagonist at specific excitatory amino acid receptors, greatly inhibits the response to ibotenate and that lithium and seizures did not alter this modulatory effect.

NaF activates phosphoinositide hydrolysis, purportedly by activation of the associated G-protein by AlF_4^-. There was no significant effect of seizures on NaF-stimulated phosphoinositide hydrolysis (Figure 30), which agrees with the findings given above that the effects of seizures were neurotransmitter-selective.

Phosphoinositide hydrolysis stimulated by NE, as well as by other agonists, is inhibited by activators of protein kinase C, such as PDBu. Therefore, the influence of seizures on this inhibitory modulation was investigated. Figure 31 shows that 1 μM PDBu inhibited the responses to carbachol and NE by 45% and 48% in control cortical slices, and by 44% and 44%, respectively, in slices prepared from rats undergoing seizures induced by lithium plus pilocarpine. PDBu (phorbol-12,13-dibutyrate)also further reduced to similar extents in control and treated samples NE-stimulated phosphoinositide hydrolysis that was partially inhibited by quisqualate. A similar lack of effect of seizures on PDBu-induced inhibition of phosphoinositide hydrolysis was observed in hippocampal slices (data not shown).

To determine if induction of seizures by another cholinergic-mediated mechanism caused similar changes, phosphoinositide hydrolysis was also measured in hippocampal and cortical slices prepared from rats in which seizures were induced by DFP (Figure 32). In both brain regions, PI hydrolysis induced by NE was reduced, although to a lesser extent than after seizures induced by lithium plus pilocarpine, but the response to carbachol, with or without 15 mM K^+, was unaffected by DFP-induced seizures.

Effects of seizures on protein kinase C

The activity of protein kinase C was determined by measuring protein phosphorylation in membrane and cytosolic fractions prepared from control or treated rats using [^{32}]ATP and the specific activators of this enzyme, calcium, PS, and the phorbol ester PMA. Protein phosphorylation catalyzed by kinases other than protein kinase C was measured in the presence of only calcium, and this background, which was typically 20-25% of total phosphorylation, was subtracted from the measurement of total phosphorylation to calculate specific phosphorylation catalyzed by protein kinase C.

In the cortex, the activity of protein kinase C in controls was 5.60 ± 0.75 and 4.17 ± 0.59 nmol/min/mg protein (n=17) in the cytosolic and membrane fractions, respectively. In the hippocampus, control protein kinase C activity was 11.47 ± 2.07 and 4.77 ± 0.75 nmol/min/mg protein (n=16) in the cytosolic and membrane fractions, respectively. Thus, in control cortex 43% of the protein kinase C was membrane-bound, while in the hippocampus this value was 29%.

Seizures were induced in rats by administration of pilocarpine (30 mg/kg; sc) to lithium-treated (3 mmol/kg; ip; 20 hours prior) rats. Assays were carried out at several times after pilocarpine treatment, including 10 minutes (prior to seizure activity), 20 minutes (coincident with the initiation of seizures), 60 minutes (after 35 to 40 minutes of status epilepticus) and 120 minutes (after approximately 100 minutes of status epilepticus). Figures 33 and 34 show the effects of seizures on cytosolic and membrane-bound protein kinase C activity in the cortex and hippocampus, respectively. In the cortex, treatment with lithium and pilocarpine and the resultant seizures caused no statistically significant changes in the protein kinase C activity in the cytosolic or membrane fractions. Thus, there was no evidence of translocation of protein kinase C to the membrane, which is sometimes taken as an indication of activation of protein kinase C. To check these results we also induced seizures with the excitatory amino acid agonist, kainic acid (10 mg/kg). With this agent we tested periods of 20 minutes (n=4; when seizure activity begins) and of 96 minutes (n=2, when status epilepticus begins). Some rats were pretreated with lithium (3 mmol/kg; ip; 20 hours prior) followed by kainic acid and assays at 20 minutes (n=4) and 96 minutes (n=2). As with the lithium and pilocarpine-induced seizures, there was little change in the protein kinase C activity in cortical cytosolic or membrane fractions after treatment with kainic acid alone or in combination with lithium. Additionally, lithium treatment alone did not alter protein kinase C activity.

In the hippocampus (Figure 34), results obtained were very similar to those observed in the cortex. Thus, there was no alteration of protein kinase C activity in the cytosolic or membrane fractions 10, 20 or 120 minutes after pilocarpine administration to lithium-treated rats.

There was an indication of decreased protein kinase C activity in both fractions 60 minutes after pilocarpine treatment, but this single alteration is difficult to explain considering the other lack of changes. Notably, treatment with lithium alone also tended to reduce the protein kinase C activity in both fractions.

As in the cortex, the results with the seizures induced by lithium and pilocarpine were checked using administration of the convulsant excitatory amino acid agonist kainic acid. Again there was little indication of any changes in protein kinase C activity. Additionally, calculation of the percent membrane-bound protein kinase C activity indicated that no significant translocation of protein kinase C to the membranes had occurred during seizures.

To ensure that our assays of protein kinase C activity were providing accurate measurements, we also employed an alternative method. With this method, the specific binding of a labelled phorbol ester ([^{3}H]PDBu) was measured in membrane preparations. This ligand binds specifically to protein C kinase, and thus measurement of its binding provides a quantitative assay of the protein kinase C present. Table 9 shows the results of the [^{3}H]PDBu binding experiments. These were carried out in membrane fractions from cortex and hippocampus from controls and from seizing rats treated previously with lithium and pilocarpine (25 or 60 minutes prior). There were no significant effects of seizures on [^{3}H]PDBu binding at either time period and in either region. Thus, these results confirm our findings using protein phosphorylation as a measure of protein kinase C activity.

Protein tyrosine phosphorylation

Although many proteins are phosphorylated on serine or threonine amino acids, several critical proteins are phosphorylated on tyrosine amino acids. Specific tyrosine phosphoproteins can be detected using antibodies directed towards the phosphotyrosine residues. Therefore, this approach was utilized to identify the effects of seizures on the phosphorylation of tyrosines in endogenous proteins in rat brain.

Figure 35 shows phosphotyrosine immunoblots from homogenates of hippocampus and cortex prepared from rats that had been sacrificed by decapitation or microwave irradiation.

Similar patterns were evident in both regions, with several prominent bands. Equivalent bands were present in samples using each method of sacrifice, indicating that microwave irradiation is appropriate for this method of analysis. Therefore, microwave irradiation was used in all further experiments (except for those in which subcellular fractions were prepared) to optimize the detection of in vivo alterations which might be transient and sensitive to postmortem alterations after decapitation.

Status epilepticus was induced in rats by administration of pilocarpine (30 mg/kg; ip) to LiCl-treated (3 mmol/kg; ip; 20 hours prior) rats. This treatment induces paroxysmal spike activity and spike trains 15 to 20 minutes after pilocarpine, followed by generalized convulsive status epilepticus which continues unabated for several hours (11). Figure 36 shows that 60 minutes after pilocarpine treatment (during status epilepticus) there was increased phosphotyrosine associated with a 40 kD protein in the hippocampus, cerebral cortex, and striatum, while, at most, only slight changes were observed in the other phosphotyrosine proteins. The increases in the tyrosine phosphorylation of the 40 kD protein resulted in values greater than threefold those of controls in all three regions.

Figure 37 shows the time course of the increased tyrosine phosphorylation of the 40 kD protein in the hippocampus of acute lithium-plus-pilocarpine-treated rats. Samples were analyzed prior to paroxysmal spiking (10 minutes), during a period of intermittent spike trains (20 minutes), immediately after the first tonic-clonic seizure which signals the initiation of status epilepticus (which averaged 25 minutes), and early or late during status epilepticus. There was an abrupt increase in the phosphotyrosine content of the 40 kD protein at a time (25 minutes) coincident with the initiation of status epilepticus, and the increase was maintained to at least 120 minutes. No significant differences were observed in the 45, 60, and 180 kD proteins, while relatively slight increases in tyrosine phosphorylation occurred in the 50 kD protein during seizures and in the 110 kD protein at 60 minutes. Similar changes were observed in the cortex, and acute lithium administration alone caused no significant changes (data not shown).

Figure 38 shows that chronic lithium treatment did not alter the extent of protein tyrosine phosphorylation in the cortex, but caused 15 to 25% reductions in the 40, 45 and 180 kD proteins in the hippocampus. Pilocarpine alone produced a 15% increase in the tyrosine phosphorylation of the hippocampal 40 kD protein, and 22 to 50% increases in the 40, 45, 50 and 110 kD proteins in the cortex. Seizures were induced by administration of pilocarpine to chronic lithium-treated rats and the phosphotyrosine proteins were analyzed 60 minutes later. As observed with seizing rats treated acutely with lithium, the major change caused by seizures was an increase in the tyrosine phosphorylation of the 40 kD protein, which was twice the control value in each region. Seizures also blocked or reversed the pilocarpine-induced increases in the phosphotyrosine content of the 45 and 110 kD proteins in the cortex.

To determine if the increase in tyrosine phosphorylation of the 40 kD protein was a response specific to coadministration of lithium and pilocarpine or if it was a general response to seizure activity, kainic acid was used to generate status epilepticus. Figure 39 shows that during kainate-induced seizures, there was a fourfold increase in the tyrosine phosphorylation of the 40 kD protein in the hippocampus, but it was only increased to 168% of control in the cortex. Kainate-induced seizures also resulted in slight increases in the tyrosine phosphorylation of the 110 kD and 180 kD proteins in the cortex.

To identify the cellular location of the 40 kD phosphotyrosine protein, cytosolic and membrane fractions were prepared from the cortex of control and seizing rats (60 minutes post-pilocarpine after acute lithium treatment) sacrificed by decapitation because microwave irradiation alters the intracellular distribution of proteins. Figure 40 shows that the majority of the 40 kD phosphotyrosine protein was located in the cytosolic fraction. Because of the absence of detectable amounts of the 40 kD band in control cytosol, it was impossible to quantitate the percent increase resulting from seizure activity.

Phosphatidylinositol hydrolysis in membranes

Measurement of [^{3}H] PI hydrolysis by membranes was used to discover whether the inhibitory effects of lithium and of seizures induced by administration of lithium and pilocarpine

to rats on phosphoinositide hydrolysis was due to impaired function of the G-protein associated with this system. Analogs of guanosine triphosphate (GTP) cannot cross membrane barriers which are present in slices, but since those are removed in membrane preparations, direct activation of G-proteins can be studied.

[^{3}H]PI hydrolysis was measured in cortical membranes incubated in the absence or presence of 8 mM LiCl to test whether lithium has a direct effect on this process (Figure 41). In control membranes (Figure 41A) in the absence of lithium, GTPγS (3 μM) induced a 95 ± 11% stimulation above basal, carbachol (1 mM) alone did not stimulate [^{3}H]PI hydrolysis, but carbachol plus GTPγS increased hydrolysis 213 ± 8% above basal and 60 ± 8% above GTPγS alone. Pilocarpine plus GTPγS stimulated [^{3}H]PI hydrolysis to the same extent as did carbachol plus GTPγS. NaF (20 mM, with 10 μM $AlCl_3$) stimulated [^{3}H]PI hydrolysis 207 ± 7% above basal. Carbachol included NaF caused no further stimulation of [^{3}H]PI hydrolysis, and NE (100 μM) alone or with GTPγS did not stimulate [^{3}H]PI hydrolysis (data not shown). Thus, both G-protein-dependent and cholinergic agonist-induced hydrolysis of [^{3}H]PI was evident with this procedure. Incubation with 8 mM LiCl did not alter the responses to any of these agents, indicating that lithium has no rapid direct inhibitory effects on these processes.

Also in cortical membranes from chronic lithium-treated rats (Figure 41B), [^{3}H]PI hydrolysis was not altered by addition of 8 mM LiCl to the incubation medium. [^{3}H]PI hydrolysis was induced by activation of G-proteins and by cholinergic agonists in the presence of GTPγS similar to the results in control cortical membranes, but the responses to agonists and to NaF were lower, indicating that a more detailed comparison would be informative.

The concentration-dependence of the stimulation by GTPγS in the absence or presence of carbachol, and by NaF on [^{3}H]PI hydrolysis in hippocampal membranes from control or chronic lithium-treated rats is shown in Figure 42. In controls, GTPγS induced a concentration-dependent stimulation which reached a plateau at approximately 3 μM and resulted in a maximal effect that was 239 ± 6% the basal activity. Addition of carbachol significantly increased hydrolysis at all concentrations of GTPγS with a maximal stimulation 339 ± 7% of basal. In

hippocampal membranes from chronic lithium-treated rats, [^{3}H]PI hydrolysis was stimulated similarly to control membranes by concentrations of GTPγS up to 10 μM, at which point it was lower. Stimulation by carbachol in the presence of 1, 3 or 10 μM GTPγS was significantly lower than controls after chronic lithium treatment. Figure 42B shows that NaF induced a concentration-dependent stimulation of [^{3}H]PI hydrolysis with a maximum that was 314 ± 4% of basal in controls and that the response in membranes after chronic lithium treatment was significantly lower than controls at all concentrations of NaF.

Results similar to those in hippocampal membranes were obtained when [^{3}H]PI hydrolysis was measured in striatal membranes from control and chronic lithium-treated rats (Figure 43). GTPγS induced a concentration-dependent stimulation of [^{3}H]PI hydrolysis with a maximal effect that was 203 ± 8% basal, and carbachol increased the hydrolysis to a maximum of 281 ± 9% of basal. Chronic lithium treatment significantly reduced the maximal response to GTPγS and the stimulation by carbachol with 1, 3 and 10 μM GTPγS. The NaF concentration-dependent hydrolysis of [^{3}H]PI in striatal membranes was similar to that obtained with hippocampal membranes, and chronic lithium treatment significantly impaired [^{3}H]PI hydrolysis at all concentrations of NaF.

The same inhibitory effects of chronic lithium treatment found in hippocampal and striatal membranes were observed in cortical membranes (using different groups of treated and control rats). Thus, lithium treatment reduced [^{3}H]PI hydrolysis induced by 10 μM GTPγS, carbachol plus 1, 3 or 10 μM GTPγS, and all concentrations of NaF (Figure 44).

In order to test the agonist-specificity of the impairment of [^{3}H]PI hydrolysis caused by lithium treatment, a series of excitatory amino acid agonists were tested for their abilities to stimulate hydrolysis of [^{3}H]PI in hippocampal membranes. Preliminary concentration-response (0.03, 0.1, 0.3, 1.0 mM) experiments demonstrated that a concentration of 1 mM of each of the effective agonists (with 3 μM GTPγS) caused the greatest stimulation of [^{3}H]PI hydrolysis (data not shown). In the presence of 3 μM GTPγS, quisqualate, trans-1-aminocyclopentyl-1-3-dicarboxylate (ACPD), kainate, and NMDA, but not glutamate (each at 1 mM), activated [^{3}H]PI

hydrolysis (Figure 45). Quisqualate and ACPD also caused a small increase in [^{3}H]PI hydrolysis in the absence of GTPγS.

Using hippocampal membranes from another group of treated and control rats, we confirmed that chronic lithium treatment reduced [^{3}H]PI hydrolysis stimulated by NaF but not by 3 μM GTPγS (Figure 46). The stimulation induced by quisqualate or by quisqualate plus 3 μM GTPγS was unaffected by chronic lithium treatment.

High concentrations of Ca^{2+} increase [^{3}H]PI hydrolysis by directly activating phospholipase C. Chronic lithium treatment did not affect the Ca^{2+} concentration-dependent hydrolysis of [^{3}H]PI in hippocampal membranes (Figure 47).

The next goal of this investigation was to extend the measurements of [^{3}H]PI hydrolysis in membranes to those obtained from rats after acute lithium treatment (3 mmol LiCl/kg, ip, 20 hours prior) and after seizures induced by administration of pilocarpine (30 mg/kg, sc, 60 minutes prior) to acute lithium-treated rats.

Figures 48, 49 and 50 show [^{3}H]PI hydrolysis in membranes from cerebral cortex, hippocampus, and striatum, respectively, of control or acute lithium-treated rats. [^{3}H]PI hydrolysis was activated by either GTPγS alone, GTPγS plus carbachol, or NaF, as used in the studies after chronic lithium treatment. The effects of acute lithium treatment were essentially identical in membranes from all three regions and were equivalent to the results obtained after chronic lithium treatment. Thus, the response to GTPγS was not altered from controls, but the responses to addition of carbachol or incubation with NaF were impaired in membranes from lithium-treated rats in all three brain regions.

Figures 51, 52 and 53 show [^{3}H]PI hydrolysis in membranes from control or seizing (acute LiCl plus pilocarpine-treated) rat cerebral cortex, hippocampus, and striatum, respectively. [^{3}H]PI hydrolysis was stimulated by the same agents as tested previously, GTPγS alone, GTPγS plus carbachol, and NaF. After seizures, the impaired [^{3}H]PI hydrolysis that was caused by lithium treatment alone was greatly attenuated. Thus, in all three brain regions, there was only a slight, if any, inhibition of [^{3}H]PI hydrolysis after incubation with GTPγS plus carbachol or with

NaF. These results show that, under these conditions, seizures increased [^{3}H]PI hydrolysis above that obtained after treatment with lithium alone to reach almost control levels of activity.

In vivo Ins 1,4,5P$_3$ concentration

The previous studies of phosphoinositide metabolism provided indirect measurements of the effects of seizures since in vitro assays were used following in vivo treatments. The recent development of a specific assay for the mass of Ins 1,4,5P$_3$ allows for the direct measurement of the production of this product from phosphoinositides. This is advantageous because it does not require the use of radioisotopes, so prelabelling steps are not required, and the entire mass, not just the labelled component, of Ins 1,4,5P$_3$ can be measured. The concentration of Ins, 1,4,5P$_3$ is of great importance, of course, because it is the primary second messenger produced by phosphoinositide hydrolysis and its major function is to release intracellular calcium from sequestered compartments.

In the following studies, the concentration of Ins, 1,4,5P$_{33}$ was measured in rat cortex and hippocampus in controls, at 20 or 60 minutes after pilocarpine (30 mg/kg, sc) administration, after acute LiCl (3 mmol/kg, ip, 20 hours prior), after chronic LiCl (4 weeks of dietary administration), or after induction of seizures by treatment with pilocarpine plus acute or chronic lithium administration. The seizing rats were studied at two time periods; at 20 minutes when seizures first began, and at 60 minutes, which was during status epilepticus.

In the hippocampus, pilocarpine administration induced a 54% increase in the concentration of Ins 1,4,5P$_3$ after 20 minutes, followed by a larger increase (110%) at 60 minutes (Figure 54). Neither acute nor chronic lithium treatment altered Ins 1,4,5P$_3$, but administration of pilocarpine to either group caused a larger increase (116% and 127%, respectively) in Ins 1,4,5P$_3$ at 20 minutes than did pilocarpine in lithium-free rats, and Ins 1,4,5P$_3$ remained elevated at 60 minutes in lithium-pretreated rats.

In the cortex, the responses to each drug alone were the same as observed in the hippocampus, but there were differences after administration of both drugs (Figure 55). Thus, pilocarpine increased the concentration of Ins 1,4,5P$_3$ by 37% at 20 minutes, and it increased

further (by 83%) at 60 minutes while neither acute nor chronic lithium treatment altered Ins 1,4,5P_3. Administration of pilocarpine to either group of lithium-treated animals caused an increase in the concentration of Ins 1,4,5P_3 at 20 minutes (by 71% with acute and 101% with chronic lithium), followed by a decrease to (acute lithium) or below (chronic lithium) control values at 60 minutes, in contrast to the lithium-free rats and to the response to both drugs in the hippocampus where there was a further increase at 60 minutes. Thus, seizures clearly activated phosphoinositide hydrolysis in vivo, leading to the rapid (20 minutes) accumulation of Ins 1,4,5P_3 in both regions, and it remained elevated in the hippocampus but became depleted in the cortex after a longer period (60 minutes) of seizures.

Neurotransmitter interactions with phosphoinositide hydrolysis

Recently a new drug became available, ACPD, which is the most selective agonist known for the excitatory amino acid receptor that is coupled to phosphoinositide hydrolysis. This is a major advantage over the previously most widely used agonist, quisqualate, which activates receptors coupled to phosphoinositide hydrolysis and also activates ion channel receptors and causes inhibitory effects on phosphoinositide hydrolysis activated by agonists of some other neurotransmitter systems. The availability of ACPD now permits investigators to carry out decisive experiments to determine how neurotransmitter systems interact to modulate phosphoinositide hydrolysis.

Maximally effective concentrations of NE and ACPD individually induced the production of similar amounts of [^{3}H]inositol monophosphate in hippocampal slices (Figure 56). When added in combination there was a synergistic interaction, resulting in 93% greater phosphoinositide hydrolysis than would have been produced by a strictly additive response to these two agonists. A synergism was also observed in cerebral cortical slices which resulted in phosphoinositide hydrolysis that was 54% greater than an additive response. In contrast, in cortical slices no potentiation by ACPD or by NE was obtained when either of these agonists was incubated in combination with 1 mM carbachol, 1 mM carbachol plus 15 mM K^+, or 50 mM K^+ (data not shown). In striatal slices, the combination of NE plus ACPD produced an amount of

[^{3}H]inositol monophosphate that was approximately additive of the responses to each agonist measured individually. This latter finding of additive responses in striatal slices confirms a previous report (63).

Table 10 shows the hippocampal slice contents of [^{3}H]inositol, [^{3}H]phosphoinositides, and [^{3}H]inositol monophosphate at the conclusion of incubations with or without agonists. Also shown are three methods of calculating agonist-stimulated phosphoinositide hydrolysis. The augmented response induced by stimulation with NE plus ACPD was not due to changes in [^{3}H]inositol content or [^{3}H]phosphoinositide synthesis and was evident independent of the method used to calculate phosphoinositide hydrolysis.

The concentration-response to NE of phosphoinositide hydrolysis in cortical slices was measured in the absence and presence of 100 μM ACPD (Figure 57). Compared with the theoretical additive responses, this combination of agonists potentiated phosphoinositide hydrolysis at all concentrations of NE tested, with potentiation ranging from 29% to 38% in these experiments. Examination of the time course in cortical slices showed that phosphoinositide hydrolysis induced by either agonist was linear for at least 60 minutes (Figure 58). The combination of NE plus ACPD induced phosphoinositide hydrolysis that was greater than additive at all time periods, with potentiation ranging from 47% to 51%. Thus, there was neither a time-lag in the appearance of the augmented response nor a down-regulation over time.

The concentration-response to ACPD measured in the absence and presence of 100 μM NE revealed a biphasic effect on phosphoinositide hydrolysis (Figure 59). With concentrations of ACPD from 3 μM to 100 μM the response to the combination of ACPD plus NE was greater than additive. However, with 1 mM ACPD there was an inhibitory interaction. This inhibition was reminiscent of the inhibition caused by quisqualate of NE-stimulated phosphoinositide hydrolysis (detailed earlier in this report).

Antagonists were used to identify the adrenergic receptor subtype involved in the interaction with ACPD-induced phosphoinositide hydrolysis (Figure 60). Propranolol, a β-adrenergic-receptor antagonist, did not alter phosphoinositide hydrolysis induced by NE, ACPD,

or both agonists together. Prazosin, an α1-adrenergic receptor antagonist, blocked the response to NE, had no effect with ACPD, and reduced phosphoinositide hydrolysis produced by the combination of ACPD plus NE to that induced by ACPD alone.

Utilization of an assay medium with a reduced concentration of Na^+ (5 mM in place of 120 mM) results in enhanced NE-induced phosphoinositide hydrolysis, as detailed earlier in this report. Lowered Na^+ augmented the response to NE by 46%, and addition of ACPD caused little further stimulation (Figure 61). This may be due to a "ceiling effect" in the rate of phosphoinositide hydrolysis as noted by other investigators studying phosphoinositide hydrolysis in brain tissue (64).

The influence of Ca^{2+} on phosphoinositide hydrolysis was investigated further by measuring the influence of verapamil, which inhibits Ca^{2+} influx, and ruthenium red, which inhibits the intracellular release of sequestered Ca^{2+} (Table 11). As previously described in this report, verapamil partially inhibited NE-stimulated phosphoinostide hydrolysis. However, verapamil did not block the synergistic interaction of NE and ACPD. The results with ruthenium red were equivocal since it greatly reduced the synergistic response in cortical slices but did not do so in hippocampal slices which display the larger augmented response.

DISCUSSION

Inhibition of phosphoinositide hydrolysis by excitatory amino acids

Glutamate inhibited [^{3}H]phosphoinositide synthesis and NE-stimulated [^{3}H]IP_1 production in rat cerebral cortical slices. Examination of selective agonists indicated that this effect was likely to be mediated by quisqualate-selective receptors. Stimulation of neuronal quisqualate-selective receptors has been shown to result in calcium influx and in mobilization of bound intracellular calcium, likely as a result of stimulation of phosphoinositide hydrolysis (35). The latter response is especially prominent in the neonatal brain, but is still evident in adults (36,37). Due to the lack of effective antagonists, it is not known if the inhibitory effects of quisqualate reported in this study are mediated by one of the receptors mediating these processes

or by another receptor. We suggested that the quisqualate-induced rise of intracellular calcium may be an intermediary step in the inhibitory effect of quisqualate in part because calcium also reduces the synthesis of [^{3}H]phosphoinositides from [^{3}H]inositol in brain slices (38), and calcium inhibits two key enzymes, phosphatidylinositol synthase and phosphatidylinositol phosphate kinase (65,66). Therefore, it was necessary to examine whether calcium mediated the inhibitory effects of glutamate or quisqualate, either directly or indirectly, such as through activation of phospholipase A_2 with release of arachidonic acid. Simultaneous examination of the effects of calcium on [^{3}H]phosphoinositide synthesis and agonist-stimulated [^{3}H]IP_1 production showed quite clearly that exogenous calcium had two opposing effects, as calcium reduced [^{3}H]phosphoinositide synthesis but promoted the production of [^{3}H]IP_1 in response to agonists. The limited responses to agonists in medium with reduced concentrations of calcium obstructed direct examination of the calcium requirement for the inhibitory response to glutamate. The results obtained, i.e., that increasing calcium resulted in increased glutamate-induced inhibition of [^{3}H]phosphoinositide synthesis and of NE-stimulated [^{3}H]IP_1 production, were consistent with the hypothesis that calcium mediates this inhibitory effect, but the dual effects of calcium limit the clarity of these results. It is noteworthy that under these conditions of both glutamate- and calcium-induced suppression of [^{3}H]phosphoinositide synthesis (Figure 1), there was not a significant inhibition of carbachol-stimulated [^{3}H]IP_1 production. This suggests several possibilities, e.g., that the relevant receptors (i.e., quisqualate-selective) may be colocalized with α1-adrenergic receptors but not with phosphoinositide-coupled muscarinic receptors, or that the regulation of adrenergic- and cholinergic-coupled phosphoinositide systems may differ.

Similarly, the experiments with verapamil were consistent with the calcium-dependence of the inhibitory effects of glutamate, since increasing concentrations of verapamil reduced glutamate-induced inhibition of NE-stimulated [^{3}H]IP_1 production. However, the direct inhibitory effects of verapamil on the response to NE (which may be due to altered calcium flux or other unidentified mechanisms) obstructed a definitive conclusion. Overall, though, consistent

effects of increased calcium were reduction of [^{3}H]phosphoinositide synthesis, enhanced production of [^{3}H]IP$_1$ in response to NE, and greater inhibition of both processes by glutamate.

We attempted to dissociate the complex actions of calcium by studying the response to agonists in slices that had been prelabelled with [^{3}H]inositol. [^{3}H]Phosphoinositide synthesis was enhanced in slices that had been prelabelled in the absence of added calcium (compared with the addition of 1.3 mM calcium), but the response to NE was severely impaired. Slices exposed continuously to calcium displayed a strong response to NE, and selective inhibitory effects by excitatory amino acids were observed. These data show again that inhibition of NE-stimulated [^{3}H]IP$_1$ production by excitatory amino acids is selectively inhibited by quisqualate-selective receptors.

The quisqualate-selective receptor mediating this inhibitory effect is apparently a different subtype than those classically studied because neither the selective antagonist CNQX nor less selective antagonists blocked the inhibition. We have previously reported that L-BOAA also appears to selectively activate this inhibitory receptor (61), if indeed this is a receptor-mediated effect, which cannot be finally concluded until an antagonist is available.

Excitatory amino acid-induced inhibition of NE-stimulated [^{3}H]IP$_1$ production in prelabelled slices raised the question of whether this could be due to inhibition of [^{3}H]phosphoinositide synthesis. Even after thoroughly washing slices prelabelled with [^{3}H]inositol, much free [^{3}H]inositol is retained within the cells which can be used for [^{3}H]phosphoinositide synthesis. To test whether [^{3}H]IP$_1$ generated by NE was dependent on continued synthesis of [^{3}H]phosphoinositides, high concentrations of unlabelled inositol were added to the medium to attempt to dilute the [^{3}H]inositol and thus block any further synthesis of [^{3}H]phosphoinositides. With 20 mM inositol, the generation of [^{3}H]IP$_1$ induced by NE was already reduced by 53% after 15 minutes. Since complete elimination of [^{3}H]phosphoinositide synthesis cannot be assured, this should be taken as a minimum estimate of the required regeneration of [^{3}H]phosphoinositides for maximal NE response. This suggests that the pool of [^{3}H]phosphoinositides coupled to the α1-adrenergic receptor is relatively small and that it can be

depleted rather quickly, leading to a diminished response to NE. Thus, although this result does not show that this is the mechanism of the inhibitory effect of amino acids, it demonstrates that such a sequence of events can occur even in prelabelled slices.

Modulation of phosphoinositide hydrolysis by arachidonic acid

There are many mechanisms by which calcium might mediate the inhibitory effects of glutamate on the phosphoinositide system. It was important to examine whether arachidonic acid might mediate this process. This hypothesis was based on the following evidence: (a) glutamate has been reported to stimulate phospholipase A_2 in brain tissue (55), possibly as a consequence of elevated intracellular calcium concentration, and (b) arachidonic acid is one of the only other effectors besides excitatory amino acids that have been reported to inhibit the synthesis of phosphoinositides (67). Although a cause-and-effect relationship cannot be established in the heterogeneous brain slice preparation, by testing the effects of arachidonic acid, we were able to confirm that it is a potential mediator of the inhibitory effects of glutamate. In fact, because arachidonic acid caused a more global, less specific, inhibitory effect than did glutamate, its effects were extremely interesting. Arachidonic acid caused a concentration-dependent reduction of [^{3}H]phosphoinositide synthesis, and [^{3}H]IP_1 produced in response to either NE or carbachol was inhibited. When the inhibition of [^{3}H]phosphoinositide synthesis was taken into account by calculating the ratio of [^{3}H]IP_1/([^{3}H]IP_1 + [^{3}H]phosphoinositides), this value remained constant throughout the arachidonic acid concentration range that was examined. This shows that the reduction of [^{3}H]IP_1 production caused by arachidonic acid was matched by the reduction of [^{3}H]phosphoinositide synthesis. This strongly suggests that the two effects are directly associated, as well as that the effect of arachidonic acid applies to [^{3}H]phosphoinositide synthesis uniformly (rather than a cell-specific effect) as might be expected from a modulator not acting through cell-specific receptors. This calculated ratio provides an interesting contrast to the same value calculated following application of quisqualate with NE, which reduced the ratio by approximately half. We postulate that this is because quisqualate does not inhibit [^{3}H]phosphoinositide synthesis globally, but its effects are restricted due to the limited

distribution of quisqualate-selective receptors which are apparently at least partly colocalized with α1-adrenergic receptors since they modulate phosphoinositide responses. However, direct evidence for colocalization remains to be obtained. Thus, [^{3}H]IP_1 produced in response to NE is reduced by quisqualate to a greater extent than is the overall [^{3}H]phosphoinositide synthesis, resulting in a reduced ratio.

An obvious test of the possibility of a connection between glutamate-activated phospholipase A_2 activity and its inhibitory effects on the phosphoinositide system would be to measure the effects of inhibitors of phospholipase A_2. However, the inhibitors that are available are known not to be specific, and we found that chloroquine, BPB and quinacrine (not shown) each inhibited NE-induced [^{3}H]IP_1 formation, possibly due to direct inhibition of phospholipase C. Alternatively, we investigated whether activation of phospholipase A_2 by melittin affected phosphoinositide metabolism and found that this agent produced a significant inhibition of [^{3}H]phosphoinositide synthesis and [^{3}H]IP_1 production. This inhibitory effect of melittin could be due to liberation of arachidonic acid, but it also could be due to other perturbations of the membrane caused by melittin.

We also attempted to examine whether the effects of arachidonic acid were direct or were mediated by a product of its metabolism. In the concentrations tested, neither inhibitors of cyclooxygenase nor of lipoxygenase blocked the inhibition by arachidonic acid, suggesting that this may be a direct effect of arachidonic acid. However, solubility limitations with these drugs limited the concentrations that could be tested (we find significant inhibitory effects of NE-stimulated [^{3}H]IP_1 production by low concentrations of ethanol and dimethyl sulfoxide [DMSO], and their effects on arachidonic acid metabolism were not measured, although similar concentrations of these drugs have been reported to effectively inhibit arachidonic acid metabolism (68).

These results indicate that the inhibitory effects of the excitatory amino acids may be mediated by activation of phospholipase A_2 and liberation of arachidonic acid. However, a direct demonstration of this relationship remains to be tested.

Modulation of phosphoinositide hydrolysis by GABA

The inh[illegible]ry neurotransmitter, GABA, had modulatory effects on phosphoinositide that in general contrasted with those of the excitatory amino acids. In agreement with previous reports (42), GABA caused little or no direct activation of [^{3}H]phosphoinositide hydrolysis, but it enhanced the response to NE, especially in hippocampal slices. Enhancement by GABA (10 mM) of [^{3}H]inositol phosphate produced in response to 1 μM NE was reported previously (42). However, we found that lower concentrations of GABA effectively enhanced the response to NE in cortical and hippocampal slices and that 300 μM GABA potentiated the response to NE throughout its effective concentration range. In cortical slices, baclofen also potentiated the response to NE, suggesting that the modulatory effect of GABA is mediated by the $GABA_B$ receptor. Baclofen did not alter the inhibitory effect of quisqualate, but it reduced that of arachidonic acid. While GABA produced a substantially enhanced response to NE, and smaller, but in some regions significant, enhanced responses to carbachol and ibotenate, it is interesting to note that GABA and baclofen have been reported to inhibit phosphoinositide hydrolysis induced by histamine (69) or serotonin (70) in cortical slices from rat and mouse, respectively.

The inhibitory effects of excitatory amino acids (a) on phosphoinositide synthesis and (b) NE-stimulated hydrolysis of phosphoinositides may provide a mechanism for enhancement of the in vivo stimulation caused by glutamate by reduction of inhibitory influences of NE in specific neuronal networks. Also, if calcium is indeed the mediator of this interaction, this may provide a protective negative feedback mechanism whereby relatively long-term increases of intracellular calcium impair further production of IP_3 and its release of bound calcium, even in the face of continued receptor activation. On the other hand, the observed effects of the excitatory amino acids may represent one of the neurotoxic consequences of these agents which eventually leads to cell death.

Thus, these results are consistent with the hypothesis that modulatory effects of excitatory amino acids on the phosphoinositide system may play an important role in neurotoxicity associated with neurodegenerative conditions, such as seizures. Furthermore, since GABA is

well known to cause inhibitory, anticonvulsive responses, the finding that GABA has opposing effects to those of the excitatory amino acids on phosphoinositide metabolism further substantiates the conclusion that modulation of this system plays an important role in seizure-induced brain damage.

Modulation of phosphoinositide hydrolysis by MK-801, an NMDA antagonist

MK-801 is one of the most widely used NMDA receptor antagonists, both as an anticonvulsant and to block brain damage induced by seizures and other insults. MK-801 caused an increase in phosphoinositide hydrolysis in a concentration-dependent manner. Although the concentration of MK-801 required to produce this effect appears relatively high, it is notable that similar concentrations of classical neurotransmitter agonists, such as carbachol, are required to produce maximal phosphoinositide hydrolysis in brain slices (10). Additionally, the maximal stimulation of phosphoinositide hydrolysis induced by MK-801 was similar to that produced by other agonists that induce this response. In contrast to the noncompetitive NMDA antagonist MK801, the competitive NMDA antagonist AP-7 had no effect on [^{3}H]-IP_1 production. Also PCP, another noncompetitive antagonist which is much less potent than NMDA, produced a relatively small stimulation and only at high concentrations. A number of drugs known to affect MK-801 binding to the NMDA receptor ion channel (71,72), including NMDA, glycine (alone or in combination), Mg^{2+}, and Zn^{2+}, did not alter the response to MK-801. Interestingly, administration of relatively high doses of MK-801 has been reported to cause seizure activity (16,73) and also vacuolization of particular neurones in the rat brain (74). Thus, there is increasing evidence that the effects of MK-801 are complex, and the findings reported here may be related to some of these poorly understood responses to MK-801. These observations are not without precedent as the competitive NMDA antagonist AP-5, at similar concentrations to those of MK-801 used in this study, was shown to act at a non-NMDA receptor site (75). Further experiments are needed to identify this novel MK-801 binding site, its properties, and distribution, but they are beyond the goals of this project. It is notable that the stimulatory effects of MK-801 on phosphoinositide hydrolysis support our previous observations and conclusions

with excitatory and inhibitory amino acids. Thus, agents which are protective towards brain damage, GABA and MK-801, each enhanced phosphoinositide hydrolysis, though clearly by different mechanisms. On the contrary, the excitatory amino acids, which appear to participate in the induction of brain damage under a variety of neurotoxic conditions, are able to inhibit agonist-induced phosphoinositide hydrolysis.

Modulation of phosphoinositide hydrolysis by Na^+

Na^+ was found to have a strong modulatory influence on phosphoinositide hydrolysis in rat brain slices, and this modulation was selective for only some of the agonists which activate this system. Most notable was the Na^+-sensitivity of the responses to quisqualate. Quisqualate can directly stimulate phosphoinositide hydrolysis, and it also inhibits NE-stimulated phosphoinositide hydrolysis (38,60). Reduction of the Na^+ concentration increased both the efficacy and the potency of the stimulatory response to quisqualate. Thus, low Na^+ converted what was a small quisqualate response requiring relatively high concentrations of quisqualate in normal Na^+ to a strong response which was greater than that of ibotenate and was evident even at a very low quisqualate concentration. The suboptimal stimulation by quisqualate of phosphoinositide hydrolysis observed in high concentrations of Na^+ suggests that Na^+ limits this interaction in many situations and that, under appropriate conditions, quisqualate can activate phosphoinositide metabolism to a much greater extent than was recognized previously. In contrast to the enhancement by low Na^+ of the stimulatory response to quisqualate, low Na^+ eliminated quisqualate-induced inhibition of NE-stimulated phosphoinositide hydrolysis. These two opposing effects of quisqualate precluded simple delineation of whether the inhibitory modulation was blocked by low Na^+ or was masked by the enhanced stimulation produced by quisqualate in low Na^+. Thus, we utilized the excitatory amino acid L-BOAA to solve this problem. L-BOAA mimics the inhibitory, but not the stimulatory, response to quisqualate (61). The finding that low Na^+ also blocked the inhibition induced by L-BOAA indicates that the two responses to quisqualate are separate processes and they are differentially influenced by the concentration of Na^+. Thus, low Na^+ enhanced the stimulation of phosphoinositide hydrolysis

induced by quisqualate and blocked its inhibitory effect on the response to NE. These results indicate that if the Na^+ concentration is altered, it will have an important influence on the interactions between quisqualate and phosphoinositide metabolism. The results with quisqualate also suggest that responses measured in 120 mM Na^+ are due to a summation of the stimulatory and inhibitory responses to quisqualate and that lowered Na^+ serves to separate these two processes, so the stimulatory response is more evident at lower quisqualate concentrations while the inhibitory effect is only observed at 1 mM quisqualate. Others have also reported that omission of Na^+ from the incubation medium reduced inhibition by NMDA of carbachol-stimulated phosphoinositide hydrolysis in hippocampal slices (76,77). Thus, physiological concentrations of Na^+ may be a common requirement for one mechanism by which excitatory amino acids inhibit agonist-stimulated phosphoinositide hydrolysis, and the selective interactions among excitatory amino acid agonists and other neurotransmitter systems may be dependent upon the receptor distributions. Glutamate also inhibited NE-stimulated phosphoinositide hydrolysis but, unlike quisqualate, this effect of glutamate was not blocked by reduced Na^+. This may be because glutamate induces inhibition by a different mechanism, such as by activation of phospholipase A_2 and subsequent inhibition of phosphoinositide hydrolysis by arachidonate (60), or because the effect of Na^+ is only evident with the more structurally restricted excitatory amino acid analogs. Since glutamate is the endogenous neurotransmitter, these inhibitory effects of glutamate may be the most relevant in this set of experiments.

The major observed effects of lowered Na^+ concentration on phosphoinositide metabolism, including increased basal hydrolysis, increased stimulatory responses to NE and quisqualate, and blocked inhibitory effects of quisqualate and L-BOAA may be due to multiple mechanisms. The binding characteristics of a number of neurotransmitter and hormone receptors are influenced by the Na^+ concentration. For example, Na^+ significantly reduced the quisqualate-sensitive [^{3}H]glutamate binding in a soluble preparation from rat adrenal (78). Other investigators have suggested that enhanced phosphoinositide hydrolysis occurring in low Na^+ may be due to Na^+/Ca^{2+} exchange resulting in increased intracellular Ca^{2+} (79). This may be

especially relevant to the increased basal rate of phosphoinositide hydrolysis in low Na^+ since increased intracellular Ca^{2+} caused by depolarizing concentrations of K^+ also increases phosphoinositide hydrolysis. However, high K^+ has little effect on phosphoinositide hydrolysis induced by NE or quisqualate (35), suggesting that an additional mechanism is required to explain the enhanced responses with these agonists. Furthermore, the response to carbachol is greatly potentiated by high K^+ but is unaffected by low Na^+, indicating that increased intracellular Ca^{2+} may not be responsible for the enhanced responses to agonists in low Na^+. Simply reducing the Ca^{2+} in the media cannot be used to test the Ca^{2+}-dependency of the effect of low Na^+ because the agonist responses are greatly impaired in the absence of Ca^{2+} in brain slices.

The present results may be due in part to modulation by Na^+ of G-proteins mediating phosphoinositide hydrolysis, as G-proteins associated with cyclic adenosine monophosphate (AMP) production have previously been shown to be influenced by Na^+. The inhibitory G-protein, G_i, associated with the cyclic AMP system has previously been reported to be Na^+-dependent, so that physiological concentrations of Na^+ are required for optimal activity of neurotransmitters or hormones which inhibit adenylate cyclase (80,81). Complex tissue- and brain region-dependent effects of Na^+ have been reported in adenylate cyclase studies and precise mechanisms of action of Na^+ are not clarified (82). Nevertheless, it is interesting to speculate that an inhibitory G-protein may be associated with some phosphoinositide systems in the brain, such as the inhibition of NE-stimulated phosphoinositide hydrolysis by quisqualate, and that such an inhibitory G-protein may be inactive in low Na^+ in analogy with the cyclic AMP system. As suggested by Duman et al. (82) Na^+ flux associated with neuronal activity may modulate second messenger activity by actions on G-proteins.

TTX blocks voltage-dependent Na^+ channels. It was found that TTX selectively inhibited carbachol-induced phosphoinositide hydrolysis while not affecting that induced by NE or quisqualate. This may signify that cholinergic muscarinic receptor responses are uniquely influenced by Na^+ channel activity in the brain.

These findings may be directly relevant in identifying the neurochemical responses to seizures induced by cholinergic agonists or other convulsants and the subsequent brain damage that occurs. Influx of Na^+ accompanies seizures, so it seems highly likely that the modulation by Na^+ of phosphoinositide hydrolysis induced by quisqualate and NE that we identified will induce altered responses of this important second messenger-inducing system.

Stimulatory modulation of phosphoinositide metabolism by an excitatory amino acid agonist

Several modulators of phosphoinositide metabolism have been identified as discussed above. Many of these are inhibitory, such as the effect of quisqualate on NE-induced phosphoinositide hydrolysis. Fewer modulators have been found which potentiate phosphoinositide hydrolysis, one example being GABA which alone has no effect, but it augments the response to NE. The identification and application of a new selective excitatory amino acid agonist, ACPD, allowed us to identify a new, very large potentiating effect on phosphoinositide metabolism. In the present study, ACPD was shown to activate phosphoinositide hydrolysis in rat hippocampal and cortical slices and to have a biphasic modulatory interaction with NE-stimulated phosphoinositide hydrolysis consisting of potentiation at most concentrations of ACPD that were tested and inhibition at the highest concentration used (1 mM). It was not surprising to find this inhibitory effect since other excitatory amino acid agonists, such as quisqualate, can inhibit receptor-activated phosphoinositide hydrolysis. It is highly likely that the inhibitions caused by high concentrations of ACPD or of quisqualate were mediated by the same processes.

On the other hand, we were surprised to observe in the present investigation a new interaction in which two agonists that activate receptors coupled to the phosphoinositide system had a large synergistic stimulatory effect when used in combination. The combination of NE plus ACPD induced hydrolysis of phosphoinositides in cortical slices that was approximately 50% greater than the calculated additive effects of each agent individually. Even more impressive was the potentiation in hippocampal slices, which was close to twice the calculated additive responses. The synergistic interaction appears to be associated with activation of the

primary receptors coupled with phosphoinositide hydrolysis. For example, the potentiation was not due to activation by NE of ß-adrenergic receptors since propranolol had no effect on the potentiated response. The interaction was completely dependent on activation of α1-adrenergic receptors since prasozin blocked NE-induced phosphoinositide hydrolysis and totally blocked the synergistic interaction of NE plus ACPD. We have shown previously that agonists acting at other excitatory amino acid receptor subtypes, such as AMPA or kainate, do not mimic this synergistic interaction between NE and ACPD (60) indicating that the metabotropic receptor mediates this effect. NMDA, which can increase intracellular Ca^{2+}, induces phosphoinositide hydrolysis only slightly, if at all, but has been reported to augment NE-induced phosphoinositide hydrolysis (Figure 8), although not nearly to the extent found with ACPD (60, 83).

The results indicated that a second messenger produced by phosphoinositide hydrolysis may mediate the synergistic interaction of NE and ACPD. One second messenger produced by phosphoinositide hydrolysis, diacylglycerol, activates protein kinase C. Activation of protein kinase C with phorbol dibutyrate induced inhibition of phosphoinositide hydrolysis (data not shown), indicating that this pathway cannot account for the augmented response observed in the presence of both agonists. Alternatively, increased intracellular Ca^{2+} may mediate the synergistic interaction, but blockade of the augmented response was only obtained with ruthenium red in the cortex. Additionally, a medium with low Na^{+}, which may increase intracellular Ca^{2+} by Na^{+}/Ca^{2+} exchange (84), mimicked the effect insofar as the response to NE was increased and no potentiation was observed. However, these observations do not clearly identify the mechanistic basis for the potentiated phosphoinositide hydrolysis with NE and ACPD. It is important to note that this is a unique interaction as far as we know. For example, no synergistic effect was obtained with carbachol in combination with either NE or ACPD.

The uniqueness of the synergistic interaction with NE and ACPD may result from colocalization of these two systems. It is noteworthy that several disparate animal treatments have been reported to result in increased phosphoinositide hydrolysis induced by NE and by excitatory amino acids, including colchicine administration (85), ischemia (86), spatial learning

(87), and long-term potentiation (88). Aronica et al. (88) suggested that the parallel increases in responsiveness of the α1-adrenergic receptor and the metabotropic excitatory amino acid receptor after several different interventions may be explained by common membrane domains and up-regulation of each system. The synergistic interaction between NE and ACPD reported here lends more support to the hypothesis that both systems share, to some extent, a common localization. It seems unlikely that all sites of receptor-coupled phosphoinositide hydrolysis for each agonist overlap, so the actual potentiation in mutually located sites is probably even greater than could be detected in heterogeneous brain slices. Recent evidence indicates that excitatory amino acid metabotropic receptors play an important role in hippocampal long-term potentiation and NE may have a modulatory effect (89). Phosphoinositide hydrolysis stimulated by ACPD and by NE is increased in rat hippocampal slices by long-term potentiation (85), and the synergism reported here may underlie some of the long-lasting changes established in this condition.

Protein kinase C in brain of control and lithium-treated rats

Methods were established to measure the activity of protein kinase C since this enzyme is believed to be regulated in concert with phosphoinositide metabolism. Two procedures were used, including measurements of the total activity of protein kinase C in membrane and cytosolic preparations from rat brain and measurements of the degree of phosphorylation of endogenous proteins by activation of protein kinase C. Initial experiments were carried out to establish the methods for these assays and to determine whether treatment with lithium alone altered these processes. Following lithium treatment, no change in either the distribution or activity of protein kinase C was detected. The enzyme distribution, which was about 40% cytosolic and 60% particulate, was comparable to previous reports of protein kinase C distribution in brain (47). The pattern of endogenous protein phosphorylation in particulate and soluble fractions corresponded closely with previous characterizations of protein kinase C and cAMP-dependent phosphorylation (90-92). Under basal conditions or with addition of calcium, no effects of chronic lithium treatment were observed.

Lithium treatment resulted in significant reductions of the protein kinase C-mediated phosphorylation of three proteins and of the cAMP-dependent phosphorylation of two proteins in the hippocampal particulate fractions. The selectivity of these changes suggests that they are not due to a global, nonspecific effect of lithium on the phosphorylation-dephosphorylation process, but may reflect specific responses to lithium treatment. The mechanisms involved and the identities of the affected proteins remain to be established. These changes may coincide with the selective effects of chronic lithium treatment on phosphoinositide hydrolysis and AMP synthesis, as the effects of lithium treatment on these second messenger systems appear to be specific for certain cells and neurotransmitter systems. Additionally, lithium treatment could influence specific protein kinase C isozymes present in brain (93). Differential effects on protein kinase C-mediated phosphorylation are certainly not without precedent, as previous studies have reported selectivity of the proteins phosphorylated by protein kinase C dependent upon the experimental conditions (94).

Examination of protein phosphorylation in hippocampal soluble fractions revealed less distinct effects of chronic lithium treatment compared with the particulate fractions. There were no significant changes of cAMP-dependent phosphorylation and a widespread, and somewhat variable, increase of protein kinase C-mediated phosphorylation of proteins in the soluble fraction following lithium treatment. The variable nature of this response may be due to inherent interindividual variations in the response to chronic lithium treatment in combination with the complex interactions that modulate protein phosphorylation and its measurement.

In summary, protein kinase C activity was measured in cytosolic and membrane preparations from rat brain. Additionally, endogenous proteins that were phosphorylated by either protein kinase C or cAMP-dependent protein kinase were identified. Lithium treatment had very limited effects on these processes.

Effects of seizures on phosphoinositide hydrolysis and protein kinase C

The results detailed previously in this report indicated that seizures may be associated with neurotransmitter-selective reduced phosphoinositide hydrolysis. Thus, seizures were induced by coadministration of lithium and pilocarpine. It was found that the ensuing seizures were associated with significant reductions of phosphoinositide hydrolysis induced by NE or ibotenate. The impairment in the response to NE was especially prominent and occurred rapidly upon induction of seizures. The response to NE was decreased most evidently, as relatively short periods of seizure activity induced by lithium plus pilocarpine or by DFP resulted in significantly impaired NE-stimulated phosphoinositide hydrolysis in hippocampal and cortical slices. Phosphoinositide hydrolysis induced by excitatory amino acids, including ibotenate in hippocampal slices and quisqualate in cortical slices, was also reduced after seizures induced by lithium plus pilocarpine. The decreases associated with seizures were not due to a generalized effect on components of the phosphoinositide system common to all neurotransmitter-coupled phosphoinositide systems, since seizures had no effect on phosphoinositide hydrolysis stimulated by carbachol, carbachol plus 15 mM K^+, or a depolarizing concentration of K^+. Also, phosphoinositide hydrolysis induced by NaF (62), which is thought to directly activate G-proteins mediating phosphoinositide hydrolysis, was not altered by seizures. It is interesting that both of the seizure models used in these experiments, administration of lithium plus pilocarpine or of DFP, initiated seizures by activation of cholinergic muscarinic receptors, but the phosphoinositide response to carbachol was unchanged while the response to NE was consistently decreased. Thus, there was a dissociation between the systems directly involved in the initiation of seizures and those affected by the seizure activity. Activation of noradrenergic systems has been shown to have anticonvulsant properties in a number of seizure models, including the lithium plus pilocarpine model (17). Reduction of this response to NE may play a role in the maintenance or severity of some seizures. Two of the known mechanisms which inhibit NE-stimulated phosphoinositide hydrolysis are activation of quisqualate-selective receptors and activation of protein kinase C. The effects of seizures induced by lithium plus

pilocarpine on both of these inhibitory processes were examined. It was found that although NE-stimulated phosphoinositide hydrolysis was reduced by seizures, both quisqualate and activation of protein kinase C with a phorbol ester induced further inhibitions which were similar to the percent inhibition that each induced in control slices. This suggests that neither of these mechanisms accounts for the reduction of NE-stimulated phosphoinositide hydrolysis that was observed in slices from seizing rats. Furthermore, Na^+ has an inhibitory influence on phosphoinositide hydrolysis induced by NE or by quisqualate (95). Reduction of the Na^+ concentration resulted in enhanced responses to each of these agonists in slices from control and seizing rats, but the responses in the samples from rats treated with lithium plus pilocarpine remained below those measured in controls. It is unlikely that the decreases in phosphoinositide hydrolysis reported here are due to lack of substrate delivery to the brain because 30 minutes of ischemia has been reported to increase, not decrease, phosphoinositide hydrolysis induced by NE or excitatory amino acid agonists in gerbil cortical slices (86).

The reduced NE-induced phosphoinositide hydrolysis observed in slices from seizing rats may provide a model to study the similar reduction in this response to NE that has been reported in human epileptic tissue (96). Reduced NE-stimulated phosphoinositide hydrolysis has also been reported in cortical slices from genetically epilepsy-prone rats (97) and after acute seizures (98). The effects of seizures on phosphoinositide hydrolysis reported here differ from those measured after kindling of rats, where the response to ibotenate was increased (99,100) and the response to carbachol as well as to NE was decreased (101). However, kindling studies usually measure effects after seizure episodes, whereas in the present study, tissue was obtained during seizures. Thus it appears that reduced phosphoinositide hydrolysis in response to NE may be a common effect of treatments which induce seizures, but alterations of the responses to other agonists vary among the seizure models and tissues examined, and the time at which tissue is examined relative to seizure activity is clearly an important variable to be considered.

These findings are consistent with our previous results. Thus, excitatory amino acids which play a prominent role in seizure-induced brain damage impair NE-stimulated

phosphoinositide hydrolysis, and this process is also impaired by in vivo seizure activity. NE is well known to have inhibitory, anticonvulsive effects, and it appears likely that inhibition of this function of NE plays an important role in seizures and the resultant brain damage. Also consistent with this conclusion are our findings that the inhibitory neurotransmitter GABA enhances NE-stimulated phosphoinositide hydrolysis. Thus, the modulation of the activity of this second messenger system appears to be closely linked with seizure activity.

In contrast to the clear effects of seizures on agonist-induced phosphoinositide hydrolysis, seizures did not alter the activity or intracellular localization of protein kinase C. This lack of effect of seizures induced by cholinergic stimulation was confirmed in two ways: (a) seizures induced by the excitatory amino acid agonist kainate also did not alter protein kinase C activity, and (b) another method to assay protein kinase C, [^{3}H]PDBu binding, also indicated no significant effect of seizures on protein kinase C.

Since increased intracellular calcium concentrations can cause translocation of protein kinase C from the cytosol to the membrane, we examined whether seizures had such an effect. No changes in the activity or the localization of protein kinase C were observed after seizures induced by lithium plus pilocarpine or by kainate. Both measurements of protein kinase C, protein phosphorylation and [^{3}H]PDBu binding, confirmed this resistance to seizures. These results indicate that protein kinase C activity and localization are unaffected by seizures and that this enzyme is unlikely to play a major role in the initiation or maintenance of seizures. This is consistent with a previous report that (^{3}H]PDBu binding in rat brain regions was unaffected by a single electroconvulsive shock (which was accompanied by a full tonic seizure), and only with repeated treatments (once daily for ten days) were there decreases in the cerebral cortex and cerebellum, while the hippocampus and striatum remained unchanged (102). It is possible that protein kinase C in discrete regions of the hippocampus or other structures may be affected, since, for example, ischemia induces changes in [^{3}H]PDBu binding only in specific regions of the hippocampus (103,104). This possibility of limited, cell-specific effects is supported by our findings of neurotransmitter-selective changes in phosphoinositide hydrolysis. Additionally,

there is increasing evidence that in some instances protein kinase C may be activated independently of translocation (105-108), and such an effect cannot be ruled out by the experiments reported here. Additionally, translocation of protein kinase C may be very transient, as, for example, translocation of protein kinase C in GH_3 cells has been reported to be reversed within 1 minute (109).

In summary, seizures elicited by lithium plus pilocarpine or by DFP had a selective inhibitory effect on phosphoinositide hydrolysis stimulated by NE or excitatory amino acids, whereas the responses to carbachol or to direct G-protein activation by NaF were unaffected. No effects of seizures on protein kinase C were detected. These alterations of phosphoinositide metabolism may play a role in the duration or severity of seizure activity.

This extensive series of experiments on protein kinase C activity clearly rules out major alterations in the activity or intracellular location of this enzyme in playing an important role in cholinergic agonist-induced seizures. It remains possible, however, that there are selective alterations in protein kinase C activity in a very restricted number of cells and that this limited change is impossible to detect over the unaltered activity of the majority of the protein kinase C present in brain regions. It is presently not possible to study protein kinase C activity after seizures in individual cells, so this possibility, though unlikely, cannot entirely be ruled out.

Protein tyrosine phosphorylation in rat brain

For a complete analysis of the effects of seizures on protein phosphorylation in rat brain, we also employed methods to specifically detect the phosphorylation of tyrosine amino acids on endogenous proteins. Although most proteins are phosphorylated on serine or threonine amino acids, the activity of several proteins which are critical for signal transduction have been shown to be modulated by phosphorylation of tyrosines.

Using immunoblots probed with a monoclonal antibody to phosphotyrosines, several protein bands containing phosphotyrosine residues were observed in rat brain. There were no major differences in the phosphotyrosine proteins that were detectable among the three regions that were studied: the cerebral cortex, hippocampus, and striatum. Most of these

phosphotyrosine proteins were unaffected by seizures induced by administration of lithium plus pilocarpine. This general lack of response emphasizes the specificity of the large increases in the tyrosine phosphorylation of the 40 kD protein that were observed. These increases did not precede seizure activity induced by lithium and pilocarpine, but occurred rapidly upon initiation of generalized convulsive status epilepticus. After the initial abrupt rise, there was little further increase, and no decrease, during continued seizures. This response was evident in all regions that were examined and occurred with two convulsive stimuli having different mechanisms of action. Thus, the increased tyrosine phosphorylation of the 40 kD protein is a specific response, and it is maintained for long periods of time during status epilepticus, whereas most phosphotyrosine proteins were unaltered by seizures.

The molecular mass and subcellular location of the responsive 40 kD phosphotyrosine protein suggests that it is the same one observed by Stratton et al. (110,111) which responded to carbachol and to protein kinase C activation in hippocampal slices and to maximal electroconvulsive shock. Those authors concluded that the specific tyrosine phosphorylation of the 40 kD protein occurred in response to phosphoinositide hydrolysis-induced activation of protein kinase C. This is consistent with other results from this laboratory in which we have found a massive stimulation of phosphoinositide hydrolysis associated with initiation of seizures induced by lithium plus pilocarpine (Jope, unpublished data). The association of this process to muscarinic receptor activation as found by Stratton et al. (110) in hippocampal slices, is also supported by the observation reported here that administration of the cholinergic agonist pilocarpine alone induced increased tyrosine phosphorylation of the 40 kD protein. The increased tyrosine phosphorylation of the 40 kD protein associated with seizures may be due to protein kinase C-induced activation of a specific tyrosine kinase as suggested by Stratton et al. (110). The tyrosine phosphorylation of other proteins was also increased by pilocarpine, notably a 50% increase in the 45 kD protein in the cortex, indicating further associations between muscarinic receptor activation and tyrosine phosphorylation.

Phosphatidylinositol hydrolysis in membranes

We took advantage of a recently developed method for measuring PI metabolism in membranes. This technique provides the opportunity to study PI hydrolysis without relying on equilibrium labelling of phosphoinositides with [^{3}H]inositol and the absence of limiting membrane barriers, as are present in brain slices, so that impermeant substances, such as GTPγS, can be utilized.

This investigation confirmed that activation of G-proteins and of coupled receptors stimulates the hydrolysis of exogenous [^{3}H]PI in brain membranes using the conditions established by Claro et al. (112) for maximal responses. Both GTPγS and NaF (in the presence of $AlCl_3$) induced concentration-dependent hydrolysis of [^{3}H]PI, probably by directly stimulating G-proteins that activate phospholipase C, thus allowing investigations of G-protein-stimulated [^{3}H]PI hydrolysis as well as that induced by receptors coupled to G-proteins. Cholinergic agonist-induced [^{3}H]PI hydrolysis was entirely dependent on the addition of GTPγS. It is interesting that in contrast to brain slices (113), pilocarpine stimulated [^{3}H]PI hydrolysis equally as well as did carbachol. As discussed by Wallace and Claro (85), this may reflect the environment of the receptor complex in each preparation or the distinction between hydrolysis of PI and of polyphosphoinositides, which are difficult to differentiate in brain slices. Unfortunately, NE caused no stimulation of [^{3}H]PI hydrolysis with these experimental conditions. Variations in the concentrations of GTPγS or cations may be required to obtain stimulation with NE or there may be fundamental differences in the receptor complexes mediating cholinergic and adrenergic responses. Several excitatory amino acid agonists, including quisqualate, ACPD, NMDA, and kainate, stimulated [^{3}H]PI hydrolysis in hippocampal membranes in the presence of GTPγS. Quisqualate was the most effective of the excitatory amino acid agonists and with GTPγS it activated [^{3}H]PI hydrolysis to a slightly greater extent than did carbachol plus GTPγS. Some of these agonists were also reported to activate [^{3}H]PI hydrolysis in cerebellar membranes in a recent abstract (114). Quisqualate and ACPD are well-known to stimulate phosphoinositide hydrolysis in brain tissue, but while stimulation by kainate

(e.g., 115) and NMDA (reviewed in 116) have been reported, they have been more variable. For unknown reasons, glutamate was not stimulatory under these conditions. Along with other methods to measure phosphoinositide metabolism, the use of membranes with exogenous substrates should provide important additional information about the modulation of this system, especially with conditions where G-protein function is perturbed.

In vitro addition of LiCl did not alter the stimulation of [^{3}H]PI hydrolysis induced by GTPγS, GTPγS plus carbachol or pilocarpine, or AlF_4^-. Therefore, the inhibition by lithium of carbachol-stimulated GTP binding observed by Avissar et al. (117) must have involved G-proteins other than those coupled to cholinergic-mediated [^{3}H]PI hydrolysis. Furthermore, these results clearly demonstrate that lithium does not have a rapid direct inhibitory effect on the cholinergic receptor, the G-protein, or phospholipase C mediating [^{3}H]PI hydrolysis, although slower direct effects occurring with chronic lithium administration cannot be ruled out. This contrasts with the AMP system in which lithium added in vitro directly inhibits agonist-stimulated AMP production (e.g., 118, 119).

Chronic lithium administration significantly attenuated [^{3}H]PI hydrolysis induced by GTPγS, carbachol plus GTPγS, or AlF_4^-. Previous studies have shown that agonist-stimulated hydrolysis of phosphoinositides in brain slices prelabelled with [^{3}H]inositol is lower after chronic lithium administration than in controls (e.g., 120). As discussed in those reports, the mechanism of this effect of lithium could not be clearly identified, and depletion of phosphoinositides and impaired G-protein function were raised as possible causative factors. In the present study, the use of exogenous [^{3}H]PI with membranes eliminated the possible influence of depletion of endogenous phosphoinositides. Therefore, the present finding of impaired [^{3}H]PI hydrolysis after in vivo lithium administration demonstrates that some mechanism other than phosphoinositide depletion must mediate this effect. The present results are consistent with chronic lithium treatment inducing an impairment of G-protein function since [^{3}H]PI hydrolysis induced by direct activation of G-proteins with GTPγS or AlF_4^- was impaired after lithium treatment. It is also possible that lithium could impair stimulation of phospholipase C by

activated G-proteins, although stimulation by calcium was unaltered after lithium administration. An inhibitory influence on G-protein function seems to be the most parsimonious explanation for these findings. Chronic lithium treatment recently was reported to reduce the mRNA for the stimulatory and inhibitory G-proteins associated with AMP production (121), raising the possibility that there may be a similar effect on G-proteins associated with phosphoinositide metabolism. Additionally, lithium may cause stable changes in the mechanisms modulating G-protein function, such as phosphorylation states (122), which are still not well understood. Chronic lithium treatment did not impair quisqualate-stimulated [^{3}H]PI hydrolysis. This may be because a different G-protein (pertussis toxin sensitive; 123, 124) mediates this response than the G-protein (pertussis toxin insensitive; 125, 126), mediating carbachol induced phosphoinositide hydrolysis. The G-protein species mediating phosphoinositide hydrolysis may provide some selectivity in the systems affected by lithium, as previously postulated (127). These data provide additional evidence that lithium administration limits, but does not block, signal transduction mediated by phosphoinositide metabolism.

Acute lithium treatment caused essentially the same inhibitory effects as did chronic lithium treatment, but administration of pilocarpine to lithium-treated rats reversed these inhibitory effects. This indicates that, during seizures, there is activation of this second messenger system, most likely through activation of cholinergic muscarinic receptors. Activation of phosphoinositide metabolism by seizures was confirmed in the following studies in whch the concentration of Ins 1,4,5P$_3$ was measured.

In vivo Ins 1,4,5P$_3$

All measurements of phosphoinositide responses in br ·n slices or membranes are limited by methodological requirements (e.g., preparation and incubation of tissue, and the use of radioisotopes) which could affect detection of in vivo changes in activity. The development of a new assay which provides measurements of the mass of Ins 1,4,5P$_3$ provided a new method to study the effects of cholinergic agonist-induced seizures on phosphoinositide metabolism. This approach has the advantage that in vivo alterations of the concentration of Ins 1,4,5P$_3$ can be

measured directly without introducing potential artifacts arising from in vitro preparations of brain tissues. However, it suffers from the inability to manipulate individually each neurotransmitter system coupled with phosphoinositide metabolism. Therefore, widespread effects can be identified, but limited changes of discrete systems may not be detected.

Pilocarpine administration caused a rapid increase in the concentration of Ins 1,4,5P_3 in rat cerebral cortex and hippocampus but neither acute nor chronic lithium treatment (without pilocarpine) had an effect. The increase after pilocarpine confirms that in vivo activation of cholinergic receptors stimulates phosphoinositide hydrolysis and leads to an accumulation of this major second messenger which serves to increase intracellular calcium concentrations.

Seizures were induced by administration of pilocarpine to lithium-treated rats. In both the cerebral cortex and the hippocampus, the initiation of seizures was associated with an increase in the concentration of Ins 1,4,5P_3. These results demonstrate directly that seizures induced by a cholinergic agonist lead to increased activation of phosphoinositide hydrolysis. Since a reduced phosphoinositide response to NE in brain slices after seizures was identified earlier in this investigation, it is most likely that the in vivo stimulation of Ins 1,4,5P_3 production is the result of activation of cholinergic and/or excitatory amino acid receptors.

Therefore, these in vivo measurements demonstrate directly that seizures induced by a cholinergic agonist increase phosphoinositide hydrolysis, which leads to increased concentrations of Ins 1,4,5P_3. Since Ins 1,4,5P_3 functions by releasing calcium from intracellular compartments, and increased intracellular calcium concentrations increase seizure-induced brain damage, it is evident that the process described here contributes to neurodegeneration caused by cholinergic agonist-induced seizures.

CONCLUSIONS

Several firm conclusions can be drawn from the studies completed, and recommendations for emphasis of further investigations can be formulated.

First, it is very evident that excitatory and inhibitory amino acid receptor agonists have important modulatory influences on phosphoinositide metabolism. The effects of the excitatory amino acids were generally opposite to those of the inhibitory amino acid GABA. This finding complements very well the knowledge that excitatory amino acids promote seizures and brain damage, whereas inhibitory amino acids are protective. The mechanisms whereby these agents act are not completely clear, but supporting evidence was obtained that the effects are mediated in part by increased intracellular calcium and possibly by production of arachidonic acid. Na^+ was also implicated as a modifying influence. However, further clarification of the precise mechanisms is needed.

It is also evident that there is a close association between cholinergic agonist-induced seizures and alteration of phosphoinositide metabolism. This is important since phosphoinositide hydrolysis is such a critical second messenger-producing system in the brain and it functions in part to modulate the intracellular concentration of calcium. Further work must be done to identify the mechanistic association between altered phosphoinositide metabolism and seizures and brain damage.

Protein kinase C activity was not sensitive to cholinergic-agonist-induced seizures. This was confirmed in a number of ways and indicates that further investigations of this system are unlikely to be productive.

In contrast, activation of tyrosine-specific kinases was discovered to be linked with seizures induced by cholinergic agonists. Phosphorylation of tyrosine amino acids is known to be an important method of modulation of the activities of several proteins critical in signal transduction. Thus, further study of this aspect of protein phosphorylation is clearly warranted.

Table 1. Comparison of inhibitory effects of arachidonic acid on [^{3}H]phosphoinositide synthesis and of [^{3}H]IP_1 production.

Rat cortical slices were incubated with [^{3}H]inositol and arachidonic acid (AA), at the concentrations indicated, with either NE (100 μM) or carbachol (2 mM). [^{3}H]Phosphoinositides and [^{3}H]IP_1 values are calculated from the data shown in Figures 5 and 7.

Ratio = ([^{3}H]IP_1/[^{3}H]IP_1+[^{3}H]phosphoinositide) x 100

Agonist	AA (μM)	% Inhibition PI	% Inhibition IP_1	Ratio
NE	-	-	-	16.7 ± 0.9
NE	10	3 ± 2	3 ± 3	16.7 ± 0.7
NE	20	10 ± 6	10 ± 8	17.4 ± 0.2
NE	50	22 ± 10	16 ± 10	17.9 ± 0.8
NE	100	28 ± 9	17 ± 9	18.8 ± 1.1
NE	200	43 ± 7	35 ± 9	18.7 ± 0.7
NE	500	55 ± 7	55 + 9	16.6 ± 0.9
Carbachol	-	-	-	7.9 ± 0.5
Carbachol	200	45 ± 2	40 ± 3	8.8 ± 0.9

Table 2. Effects of agents which alter phospholipase A_2 activity on [^{3}H]phosphoinositide synthesis and [^{3}H]IP_1 production.

Cortical slices were incubated simultaneously with [^{3}H]inositol, 100 μM NE where indicated (+), 500 μM quisqualate (QA) where indicated (+), and the indicated agents (final concentrations were 100 μM, except for melittin which was 20 μg/ml) for 60 min, and [^{3}H]phosphoinositide synthesis and [^{3}H]IP_1 production were measured as described in Methods. Each value represents the mean ± SE of two experiments measured in triplicate. (BPB = bromphenacyl bromide). Ratio = ([^{3}H]IP_1/[^{3}H]IP_1 +[^{3}H]phosphoinositides) x 100.

Addition	NE	QA	[^{3}H]phosphoinositide (cpm x 10^{-3})	[^{3}H]IP_1 (cpm x 10^{-3})	Ratio
-	-	-	31.5 ± 0.9	0.9 ± 0.1	2.8
-	+	-	32.8 ± 1.4	7.8 ± 0.4	19.1
Melittin	+	-	15.2 ± 0.9	3.8 ± 0.2	20.1
Chloroquine	+	-	25.3 ± 0.6	4.8 ± 0.1	15.9
BPB	+	-	21.7 ± 1.7	4.2 ± 0.3	16.4
Dexamethasone	+	-	26.9 ± 1.1	7.1 ± 0.2	20.8
-	+	+	17.1 ± 0.7	1.5 ± 0.1	7.8
Chloroquine	+	+	14.6 ± 0.6	1.0 ± 1.0	6.5
BPB	+	+	13.4 ± 1.0	1.2 ± 0.1	8.3
Dexamethasone	+	+	14.8 ± 1.0	1.4 ± 0.1	8.8

Table 3. Comparison of inhibitory effects on [^{3}H]phosphoinositide synthesis and on Mn^{2+}-activated incorporation of [^{3}H]inositol into lipids by the base exchange reaction.

Rat cortical slices were incubated with [^{3}H]inositol, and where indicated, 100 μM NE, 1 mM glutamate (Glu), 0.5 mM quisqualate (QA), or 200 μM arachidonic acid (AA), in the absence (control) or presence of 1 mM $MnCl_2$ (to stimulate the base exchange reaction) for 60 min followed by measurement of [^{3}H]phosphoinositides as described in Methods. Values are means ± SE of three experiments, each measured in triplicate. *$p < 0.05$ compared with the value obtained with NE.

	[^{3}H]Phosphoinositide			
Addition	Control (cpm x 10^{-3})	Percent Inhibition	$MnCl_2$ (cpm x 10^{-3})	Percent Inhibition
NONE	26.6 ± 1.1	-	434.2 ± 16.5	-
NE	29.6 ± 1.7	-	445.0 ± 14.1	-
NE + Glu	15.0 ± 0.8*	48	399.6 ± 18.0	10
NE + QA	19.2 ± 0.9*	35	411.8 ± 12.7	7
NE + AA	10.6 ± 0.7*	64	357.1 ± 18.9*	20

Table 4. NE-stimulated [^{3}H]IP_1 production in the presence of various modulators.

Cortical slices were prelabelled with [^{3}H]inositol for 60 minutes followed by several washes. Prelabelled slices were then incubated for 60 minutes in the presence of NE (100 μM) or NE and quisqualate (QA; 500 μM) in the absence or presence of furosemide (500 μM), diisothiocyanotostilbene-2,2-disulfonic acid (DIDS; 500 μM), or α-difluoromethylornithine (DFMO; 10 mM), and [^{3}H]IP_1 was measured. The results are expressed as [^{3}H]IP_1 formed as a percentage of total [^{3}H]. Each value represents the mean ± SE of two experiments measured in triplicate.

Addition	NE	QA	[^{3}H]IP_1
-	-	-	0.3 ± 0.1
-	+	-	3.2 ± 0.3
Furosemide	+	-	3.8 ± 0.2
DIDS	+	-	4.0 ± 0.2
DFMO	+	-	4.1 ± 0.3
-	+	+	1.5 ± 0.1
Furosemide	+	+	1.2 ± 0.1
DIDS	+	+	1.8 ± 0.1
DFMO	+	+	1.0 ± 0.1

Table 5. Protein kinase C activity in soluble and particulate fractions from hippocampus of control and chronic lithium-treated rats.

Protein kinase C activity was measured as described in Methods, and was calculated as the difference between activity measured in the presence of 1.5 mM $CaCl_2$ from that observed in the presence of $CaCl_2$,10 μg PS, and 1 μM PMA. Results are expressed as means ± SEM (n = 8-9) of quadruplicate determinations.

Fraction	Specific Activity (nmol/min/mg protein)		Total Activity (nmol/min)		Distribution (%)	
	Control	Lithium	Control	Lithium	Control	Lithium
SOLUBLE	41 ± 2	36 ± 2	75 ± 7	74 ± 8	39%	39%
PARTICULATE	15 ± 1	14 ± 1	116 ± 4	113 ± 6	61%	61%

Table 6. Effects of chronic lithium treatment on protein phosphorylation mediated by protein kinase C or cAMP-dependent kinase in the hippocampal particulate fraction.

Phosphorylation of particulate proteins was measured as described in Methods. Phosphorylation of proteins after chronic lithium treatment is expressed as the percent of control values (peak height of lithium-treated/peak height of control x 100) after subtracting phosphorylation observed in the presence of calcium alone for protein kinase C or theophylline alone for cAMP-dependent phosphorylation. (Mean ± SEM; n = 5-9 for protein kinase C, n = 8-10 for cAMP; *$p < 0.05$, Wilcoxon signed-rank test).

PROTEIN KINASE C		cAMP-DEPENDENT KINASE	
PHOSPHOPROTEIN MOLECULAR MASS (kD)	PERCENT CONTROL	PHOSPHOPROTEIN MOLECULAR MASS (kD)	PERCENT CONTROL
16	101 ± 2	47	97 ± 14
18	84 ± 6*	54	92 ± 3*
19	94 ± 2*	71	87 ± 6*
45	96 ± 7	76	87 ± 5
76	92 ± 9	138	88 ± 7
87	81 ± 6*	260	92 ± 5
158	99 ± 9		
200	101 ± 8		

Table 7. Effects of chronic lithium treatment on protein kinase C-mediated protein phosphorylation in the hippocampal soluble fraction.

Phosphorylation of soluble proteins mediated by protein kinase C was measured as described in the legend to Figure 3 and Methods. Phosphorylation of proteins after chronic lithium treatment is expressed as the percent of control values (peak height of lithium-treated/peak height of control X 100) after subtracting phosphorylation observed in the presence of calcium alone. (Mean ± SEM; n = 7 - 8; **p < 0.05; *p = 0.06, (n = 5); Wilcoxon signed-rank test.

PHOSPHOPROTEIN MOLECULAR MASS (kD)	PERCENT CONTROL
16	156 ± 24**
17	225 ± 34**
20	203 ± 29**
22	240 ± 35*
24	188 ± 43
26	141 ± 27
28	145 ± 30
30	148 ± 33
36	160 ± 43
43	100 ± 17
45	120 ± 15
50	9[illegible] ± 7
56	102 ± 26
71	119 ± 14
80	106 ± 4
250	94 ± 8

Table 8. Effects of modulators on phosphoinositide hydrolysis.

Hippocampal slices prelabelled with [^{3}H]inositol were incubated with (NE, 100 μM) in the absence or presence of quisqualate (QA, 300 μM) or with ibotenate (IBO, 1 mM) in the absence or presence of AP-4 (1 mM). QA and AP-4 inhibited each response similarly in each treatment group. The number of experiments measured in triplicate are indicated in parentheses.

	Phosphoinositide Hydrolysis (% Inhibition)		
	Control	Lithium	Li + Pilocarpine (25 min)
NE + QA	67 ± 6 (4)	71 ± 2 (3)	71 ± 9 (4)
IBO + AP-4	82 ± 4 (3)	91 ± 7 (2)	88 ± 2 (3)

Table 9. Protein kinase C associated with membranes as measured by [^{3}H]PDBu binding.

Membranes were prepared from the cortex and hippocampus of rats, including controls and rats given LiCl (3 mmol/kg; ip; 20 hours prior) and pilocarpine (Pilo) (25 or 60 minutes prior to sacrifice). The treated rats were in the initial stages of seizures (25 minutes) or were undergoing status epilepticus (60 minutes). Means ± SEM (n=3 per group).

[^{3}H]PDBu Bound

	Control	Li + Pilo (25 min)	Li + Pilo (60 min)
		B_{max} (pmol/mg protein)	
Hippocampus	33.6 ± 2.3	35.4 ± 5.2	39.1 ± 4.1
Cortex	36.6 ± 3.2	36.3 ± 7.0	35.5 ± 4.6
		K_D (nM)	
Hippocampus	0.99 ± 0.10	1.02 ± 0.10	1.13 ± 0.10
Cortex	1.30 ± 0.20	1.45 ± 0.20	0.97 ± 0.20

Table 10. Distribution of [^{3}H]inositol in hippocampal slices

	Basal	ACPD	NE	NE + ACPD	% potentiation
[^{3}H]inositol (cpm)	33148 ± 4282	30148 ± 2914	29967 ± 1936	25693 ± 2292	-
[^{3}H]phosphoinositides (cpm)	15455 ± 1867	16114 ± 1454	16482± 1144	12750 ± 1250	-
[^{3}H]InsP$_1$ (cpm)	297 ± 36	1783 ± 197	2004 ± 169	6001 ± 846	79%
$\frac{[^3\text{H}]\text{InsP}_1 \times 100}{[^3\text{H}]\text{InsP}_1 + [^3\text{H}]\text{phosphoinositides}}$	1.9 ± 0.2	10.0 ± 0.6	10.8 ± 1.1	32.0 ± 1.0	88%
$\frac{[^3\text{H}]\text{InsP}_1 \times 100}{\text{Total } [^3\text{H}]}$	0.6 ± 0.1	3.7 ± 0.2	4.2 ± 0.3	13.5 ± 0.9	93%

Slices were preincubated, labelled with [^{3}H]inositol for 1 hour, and incubated another 1 hour in the absence (Basal) or presence of 100 μM ACPD, 100 μM norepinephrine (NE), or NE plus ACPD, and radioactivity in each fraction was measured as described in the Methods. Potentiation was calculated by subtracting the basal value from each result and comparing the measured response to NE plus ACPD to that calculated by adding the individual responses to NE and ACPD. Means ± SEM (n=8).

Table 11. Effects of verapamil and ruthenium red on phosphoinositide hydrolysis.

		NE	ACPD	NE + ACPD	Theoretical additivity	% potentiation
CORTEX						
	control	2.7 ± 0.2	0.6 ± 0.1	4.9 ± 0.3	3.3	48%
	verapamil	1.3 ± 0.3	0.7 ± 0.2	2.8 ± 0.6	2.0	40%
	ruthenium red	2.6 ± 0.3	0.8 ± 0.2	3.9 ± 0.4	3.4	15%
HIPPOCAMPUS						
	control	2.7 ± 0.4	1.6 ± 0.3	8.4 ± 1.1	4.3	95%
	verapamil	1.1 ± 0.3	2.5 ± 0.2	6 0 ± 0.5	3.6	67%
	ruthenium red	2.5 ± 0.5	2.2 ± 0.4	8.3 ± 1.1	4.7	77%

Slices were preincubated, labelled with [^{3}H]inositol for 1 hour, and incubated for another 1 hour in the absence (basal) or presence of 100 μM norepinephrine (NE), 100 μM ACPD, or both agents, with 100 μM verapamil or 100 μM ruthenium red, as indicated. Potentiation was calculated by subtracting the basal value from each result and comparing the measured response to NE plus ACPD to that calculated by adding the individual responses to NE and ACPD. Means ± SEM (n=3-4).

Figure 1. Calcium dependence of [^{3}H]phosphoinositide synthesis and hydrolysis.

Rat cerebral cortical slices were incubated simultaneously with [^{3}H]inositol and the indicated agents for 60 min, with the incubation media containing: EGTA (1 mM) without added calcium; no added calcium; 0.1 mM calcium; or 1.3 mM calcium. With each incubation condition, the slices were treated with no addition (basal), NE (100 μM), NE and glutamate (500 μM), carbachol (2 mM), or carbachol and glutamate (500 μM). [^{3}H]Phosphoinositides (Figure A) and [^{3}H]IP$_1$ (Figure B) were measured as described in Methods. Each value represents the mean ± SE of three experiments measured in triplicate. For basal, NE and carbachol, each increase of the calcium in the incubation medium produced significantly ($p < 0.05$) increased production of [^{3}H]IP$_1$ and significantly decreased synthesis of [^{3}H]phosphoinositides. *$p < 0.05$ indicates significant effects of glutamate compared with the results with each agonist in the absence of glutamate.

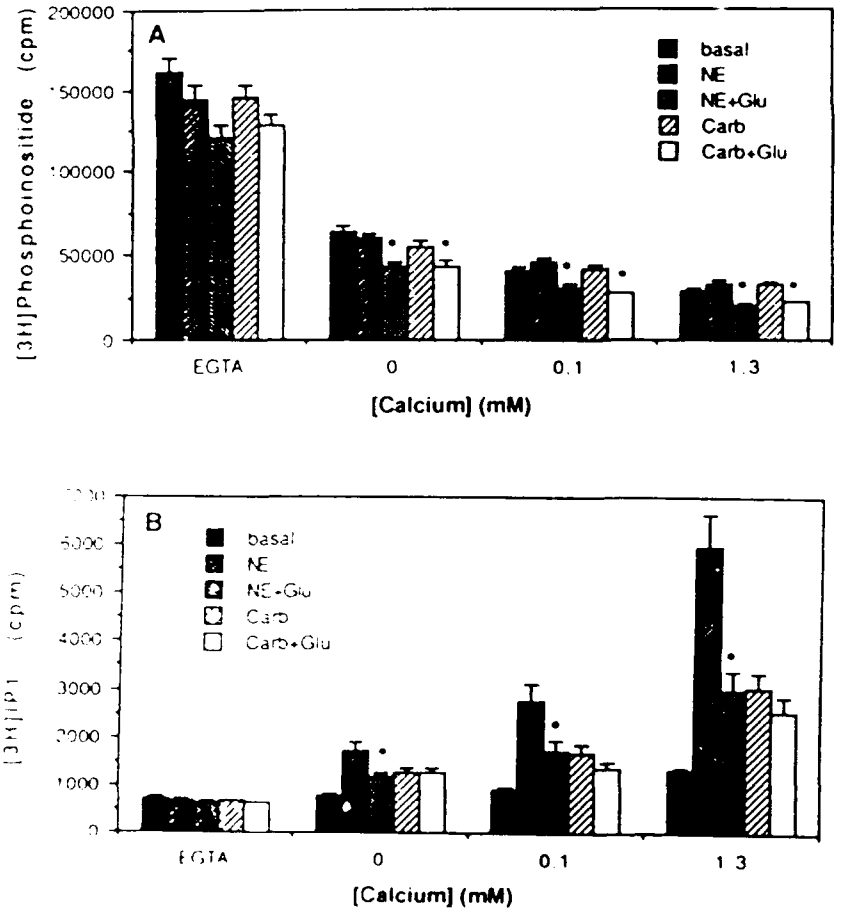


Figure 2. Effects of verapamil on [^{3}H]phosphoinositide synthesis and hydrolysis.

Rat cortical slices were incubated simultaneously with [^{3}H]inositol and verapamil (0-100 μM) for 10 min followed by 60 min incubation with no addition (□), NE (100 μM) (○) or NE (100 μM) plus glutamate (500 μM) (●). [^{3}H]Phosphoinositides (Figure A) and [^{3}H]IP_1 (Figure B) were measured as described in Methods. Each value represents the mean ± SE of three experiments measured in triplicate. All values obtained with NE plus glutamate were significantly ($p < 0.05$) less than those obtained with NE, except for [^{3}H]IP_1 in the presence of 75 or 100 μM verapamil.

*$p < 0.05$ indicates significant effects of verapamil compared with the results obtained in the absence of verapamil.

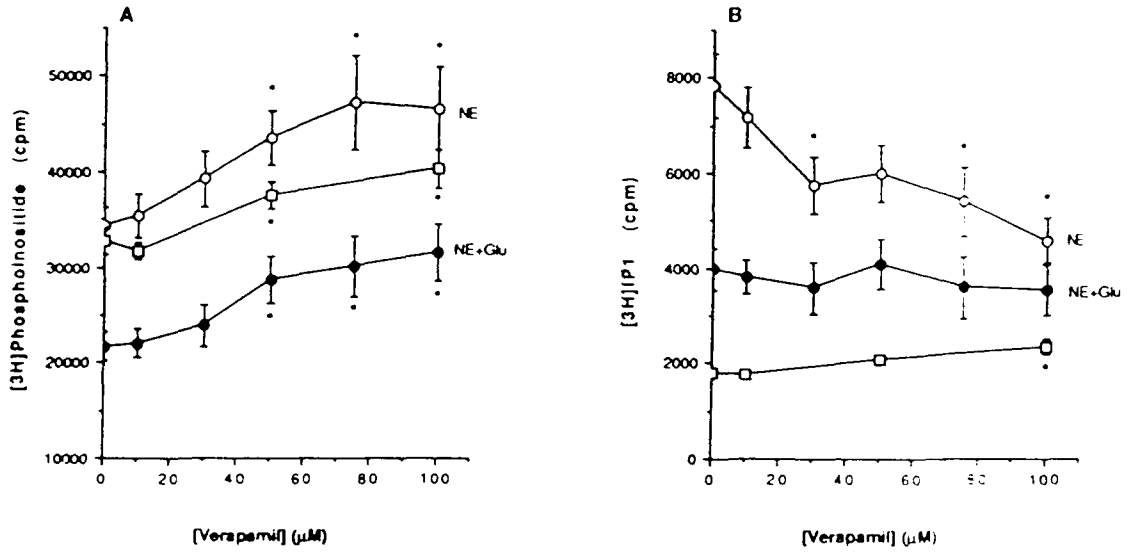
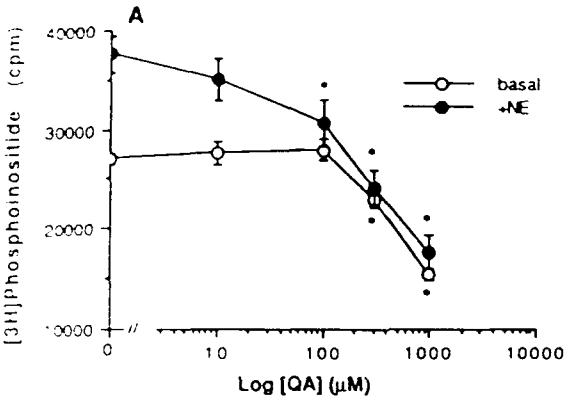


Figure 3. Quisqualate concentration-dependent inhibition of [^{3}H]inositol synthesis and hydrolysis.

Rat cortical slices were incubated simultaneously with [^{3}H]inositol and quisqualate (○) or NE (200 μM) and quisqualate (●) for 60 min. [^{3}H]Phosphoinositides (Figure A) and [^{3}H]IP_1 (Figure B) were measured as described in Methods. Each value represents the mean ± SE of three experiments measured in triplicate.

*$p < 0.05$ compared with the response in the absence of quisqualate.



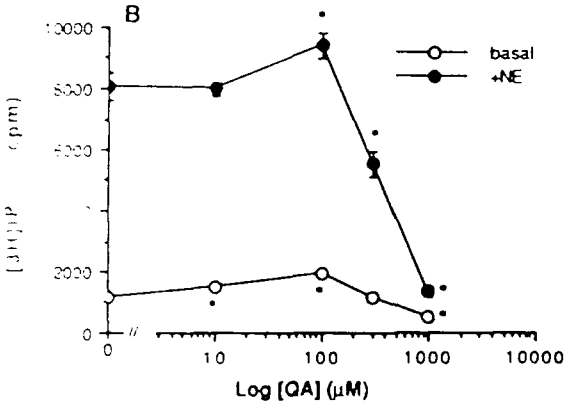


Figure 4. Excitatory amino acid antagonists do not modify quisqualate-inhibited [^{3}H]phosphoinositide hydrolysis.

Rat cortical slices were incubated with [^{3}H]inositol for 60 min and antagonists, including 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 500 μM), glutamate diethyl ether (GDEE; 500 μM), r-glutamylglycine (γ-GG; 500 μM), and DL-2-amino-4-phosphonobutyric acid (AP_4; 500 μM), for the final 10 min, followed by addition of NE (100 μM), NE and quisqualate (QA) (500 μM), or NE and AMPA (500 μM), and a further 60 min incubation period. [^{3}H]IP_1 was measured as described in Methods. Each value represents the mean ± SE of two experiments measured in triplicate.

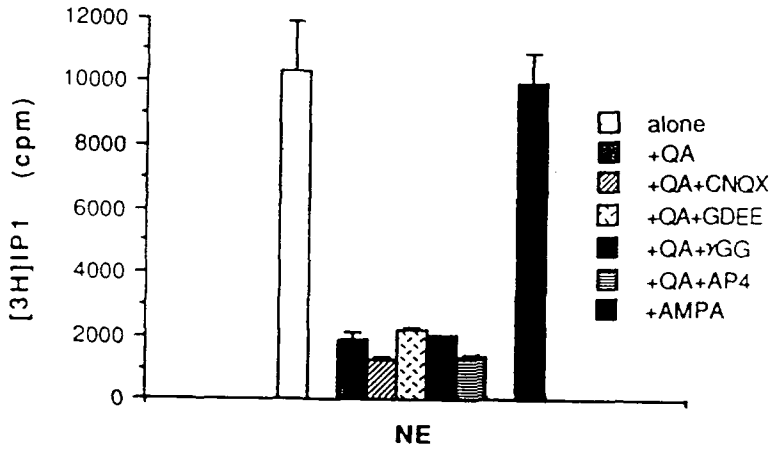
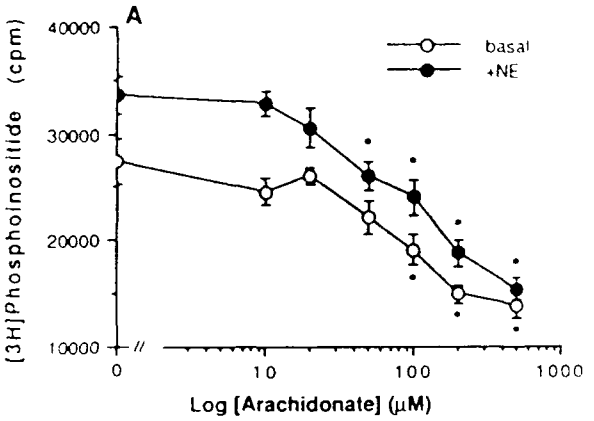


Figure 5. Concentration-response of arachidonic acid on [^{3}H]phosphoinositide synthesis and hydrolysis.

Rat cortical slices were incubated simultaneously with [^{3}H]inositol and arachidonic acid (○), or NE (100 μM) and arachidonic acid (●) for 60 min. [^{3}H]Phosphoinositides (Figure A) and [^{3}H]IP_1 (Figure B) were measured as described in Methods. Each value represents the mean ± SE of three experiments measured in triplicate. * $p < 0.05$ compared with the response in the absence of arachidonic acid.



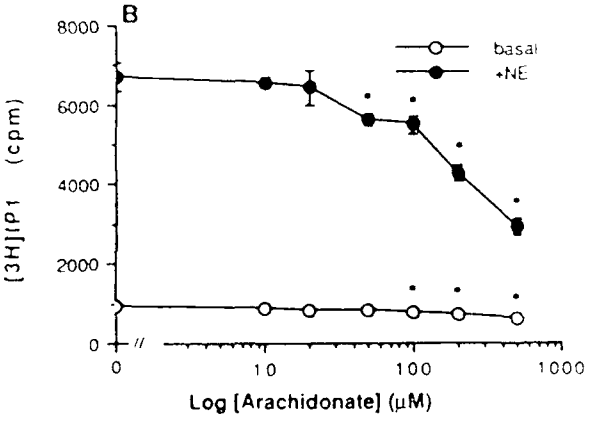


Figure 6. Time course of the effects of arachidonic acid on [^{3}H]phosphoinositide synthesis and hydrolysis.

Rat cortical slices were incubated simultaneously with [^{3}H]inositol and no addition (basal: □), 100 μM NE (○), or 100 μM NE plus 200 μM arachidonic acid (AA: ●), for 5, 10, 20, 40 or 60 min. [^{3}H]Phosphoinositides (Figure A) and [^{3}H]IP_1 (Figure B) were measured as described in Methods. Each value represents the mean ± SE of three experiments measured in triplicate. *$p < 0.05$ indicates significant differences in samples incubated with NE and arachidonic acid compared with those incubated with NE.

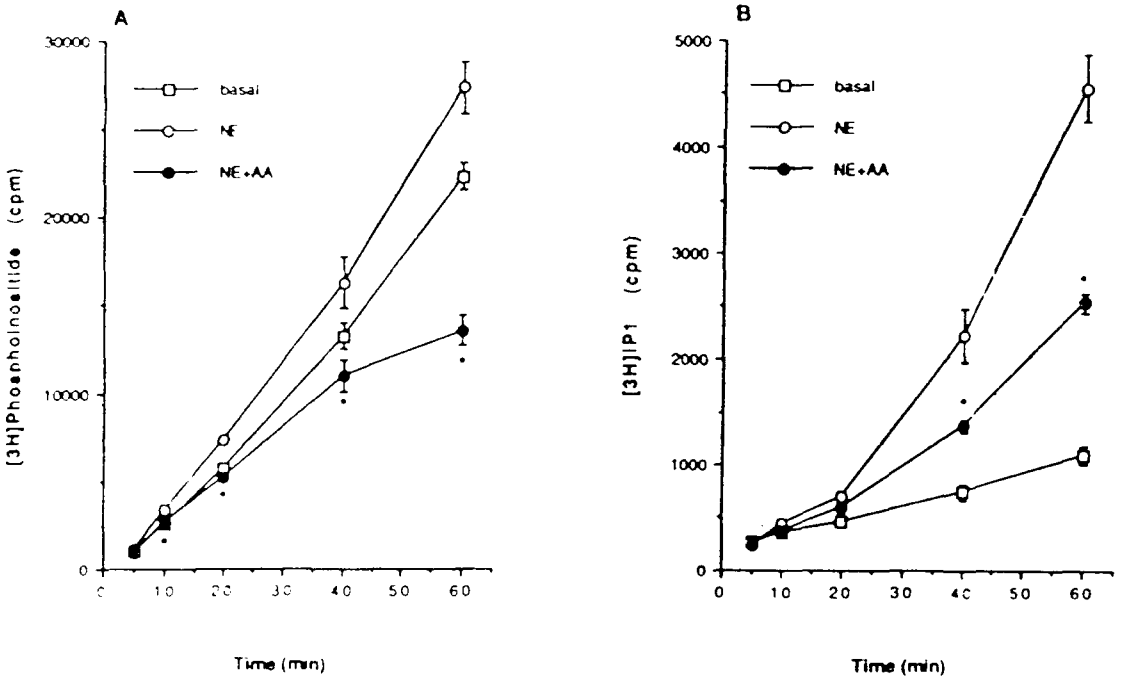


Figure 7. Arachidonic acid inhibits carbachol-stimulated [^{3}H]phosphoinositide hydrolysis.

Rat cortical slices were incubated simultaneously with [^{3}H]inositol and no addition (B; basal), carbachol (2 mM; Carb), or carbachol plus arachidonic acid (200 μM; AA) for 60 min. [^{3}H]Phosphoinositides (Figure A) and [^{3}H]IP_1 (Figure B) were measured as described in Methods. Each value represents the mean ± SE of three experiments measured in triplicate. *$p < 0.05$ compared with the value with carbachol alone.

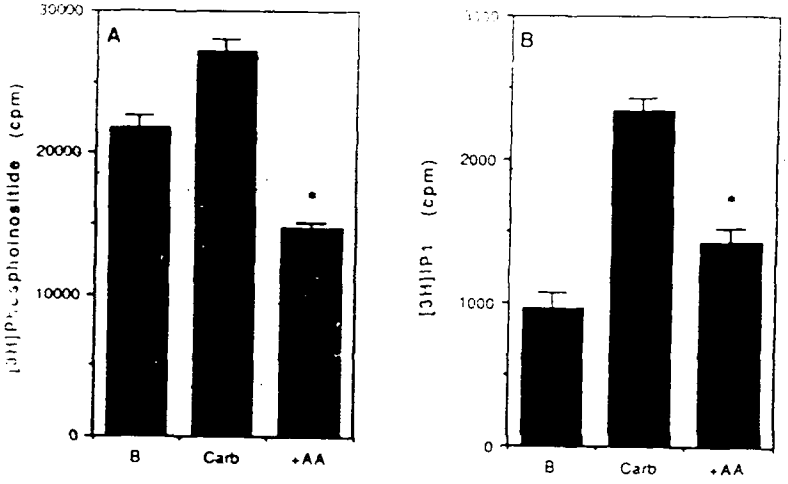


Figure 8. The effects of amino acids on NE-stimulated [^{3}H]IP$_1$ production in cortical slices that were prelabelled with [^{3}H]inositol in the presence of 1.3 mM Ca^{2+} or in the absence of added Ca^{2+}.

Rat cortical slices were prelabelled with [^{3}H]inositol for 60 min with the incubation media containing 1.3 mM calcium (+Ca) or no calcium (-Ca). After prelabelling, the slices were washed thoroughly with incubation media containing 1.3 mM calcium, and then incubated with 200 µM NE and the indicated additions for 60 min in the calcium-containing media. The left panel shows the results from slices prelabelled in calcium-containing media. The right panel shows the results from slices prelabelled in media without added calcium. The results are expressed as [^{3}H]IP$_1$ formed as percentage of total [^{3}H] (which was calculated as [^{3}H]inositol + [^{3}H]phosphoinositides + [^{3}H]IP$_1$). Each value represents the mean ± SE of three experiments measured in triplicate. *$p < 0.05$ compared with NE. HCA = homocysteic acid, CSA = L-cysteine sulfinic acid, CYS = cysteine.

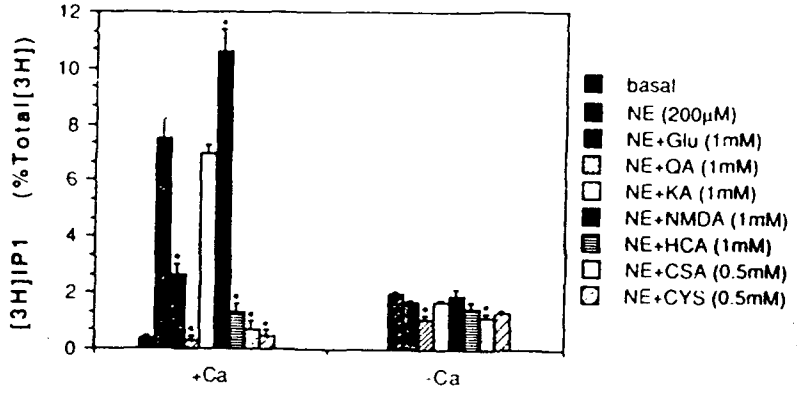


Figure 9. Inhibition by quisqualate of NE-induced [^{3}H]IP$_1$ production.

Cortical slices were prelabelled with [^{3}H]inositol for 60 min and washed, as described in the Methods. Slices were incubated for 60 min with the indicated concentrations of NE in the absence (○) or presence (●) of 500 μM quisqualate. Values are means ± SE of three experiments measured in triplicate. Quisqualate significantly ($p < 0.05$) reduced the production of [^{3}H]IP$_1$ at all concentrations of NE.

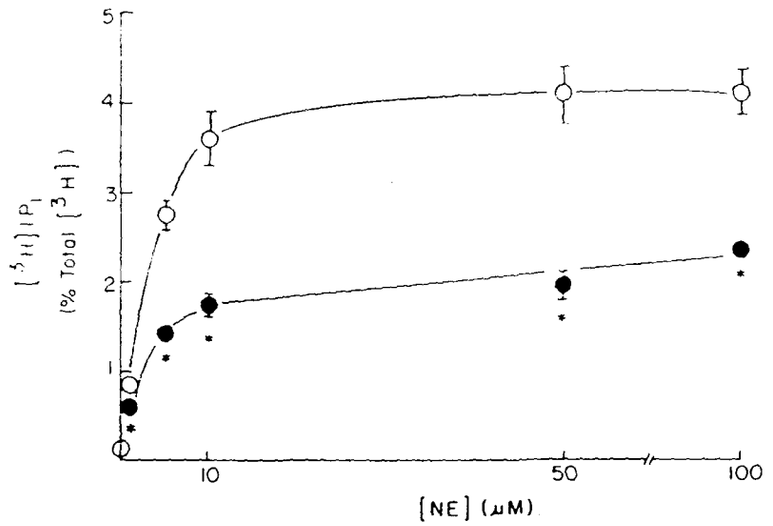


Figure 10. Incubation with unlabelled inositol reduces NE-stimulated [^{3}H]IP$_1$ production.

Cortical slices were prelabelled with [^{3}H]inositol for 60 min and washed, as described in the Methods. Slices were incubated for 15 min in incubation medium containing 0, 1, 5, or 20 mM inositol followed by addition of NE (200 μM) and incubation of 15 min (open bars) or 30 min (solid bars) and measurement of [^{3}H]IP$_1$. Values are given as % of [^{3}H]IP$_1$ produced by NE in the absence of unlabelled inositol, which were 1608 ± 308 cpm at 15 min and 3562 ± 430 cpm at 30 min (mean ± SE of 3 experiments carried out in triplicate). *$p < 0.05$

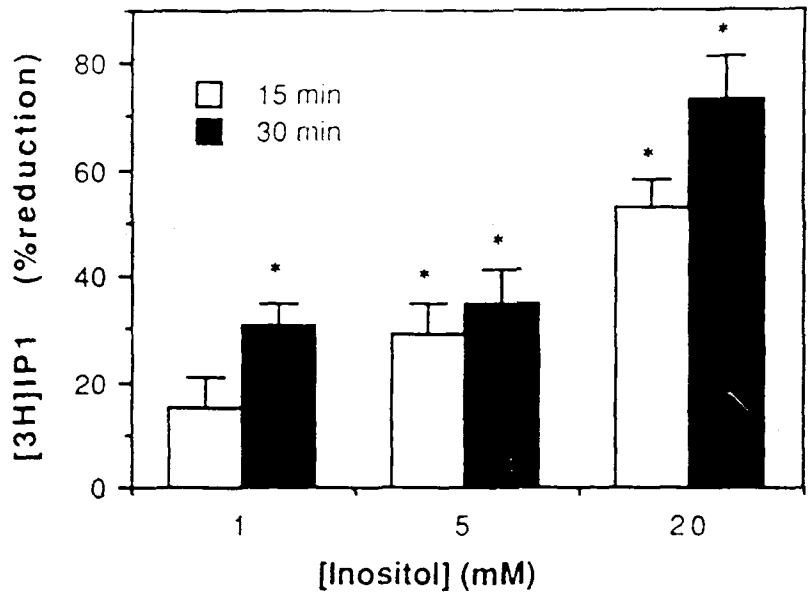


Figure 11. Effects of GABA on agonist-stimulated [^{3}H]phosphoinositide hydrolysis in cortical slices.

Cortical slices were prelabelled with [^{3}H]inositol for 60 min followed by several washes. The labelled slices were incubated for 60 min with the indicated concentrations of GABA and 2 μM NE (□), 200 μM NE (○), 200 μM NE plus 1 mM glutamate (●), or 2 mM carbachol (Δ), followed by measurement of [^{3}H]IP$_1$. Each value is the mean ± SEM of 3-5 experiments. *$p < 0.05$ compared with no GABA.

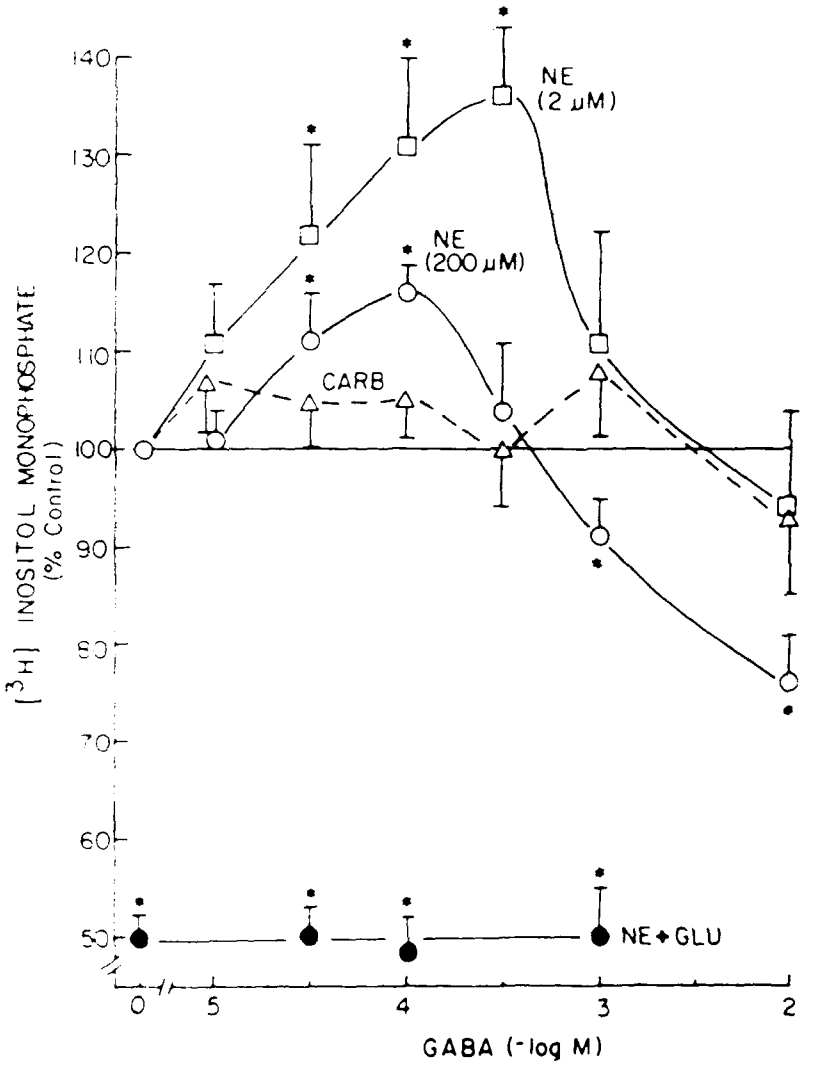


Figure 12. GABA enhances carbachol-stimulated [^{3}H]IP$_1$ production in striatal slices.

Rat striatal slices were prelabelled with [^{3}H]inositol for 60 min and then thoroughly washed. The labelled slices were incubated with 0, 100 μM, or 1 mM GABA and 200 μM NE, 2 mM carbachol, or 1 mM ibotenate (IBO) for 60 min and [^{3}H]IP$_1$ was measured as described in Methods. Values are given as the % of [^{3}H]IP$_1$ produced by each agonist in the absence of GABA, means ± SE of three experiments each measured in triplicate. *$p < 0.05$ compared with no added GABA.

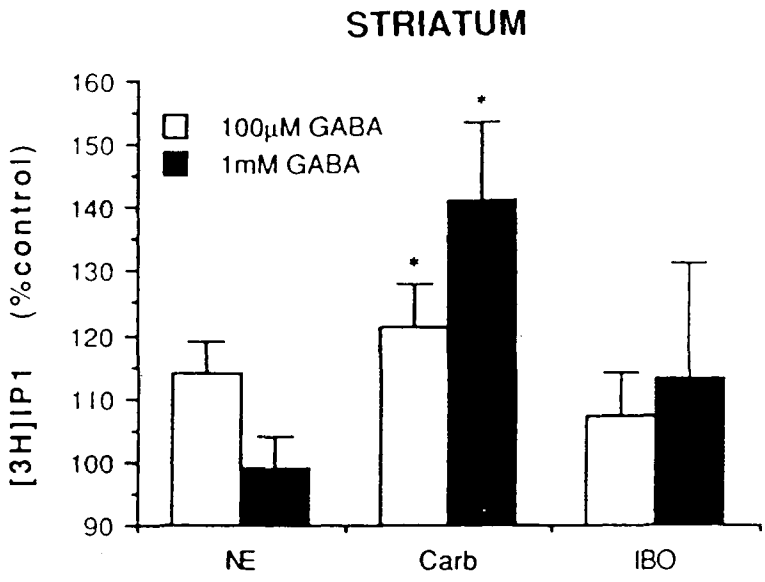


Figure 13. Modulation by GABA of agonist stimulated $[^3H]IP_1$ production in hippocampal slices.

Rat hippocampal slices were prelabelled with $[^3H]$inositol for 60 min followed by several washes. The labelled slices were incubated for 60 min with the indicated concentrations of GABA and 2 mM carbachol, 1 mM ibotenate (IBO), or 200 μ NE, and $[^3H]IP_1$ was measured as described in Methods. Values are given as the % of $[^3H]IP_1$ produced by each agonist in the absence of GABA, means ± SE of three experiments measured in triplicate. *$p < 0.05$ compared with no added GABA.

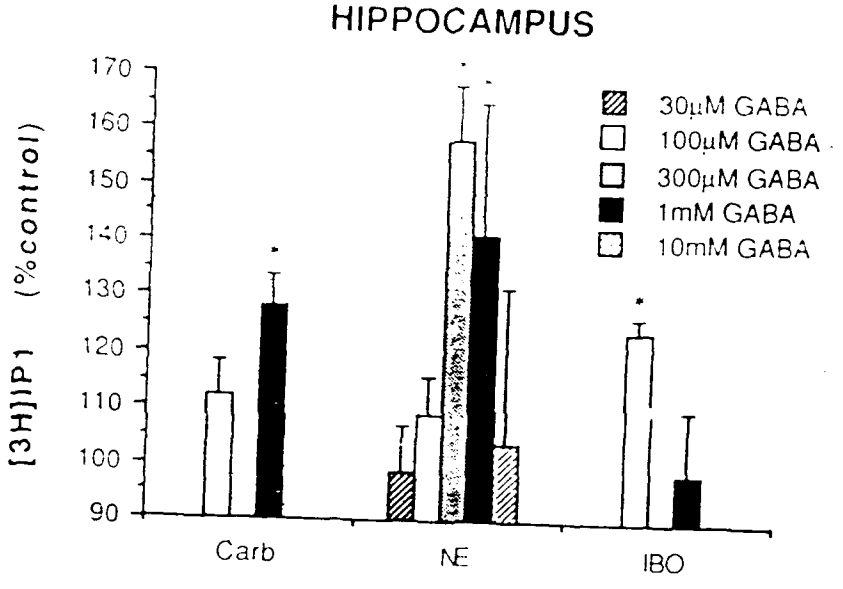


Figure 14. Potentiation by GABA of NE-stimulated [^{3}H]IP$_1$ production in hippocampal slices.

Rat hippocampal slices were prelabelled with [^{3}H]inositol for 60 min followed by several washes. The labelled slices were incubated with the indicated concentrations of NE and either 300 μM GABA (●) or no added GABA (○), for 60 min and [^{3}H]IP$_1$ was measured as decribed in Methods. Values are given as [^{3}H]IP$_1$ produced as percent of the total [^{3}H] in the slices, means ± SE of three experiments measured in triplicate. All results with GABA were significantly ($p < 0.05$) greater than those in the absence of GABA.

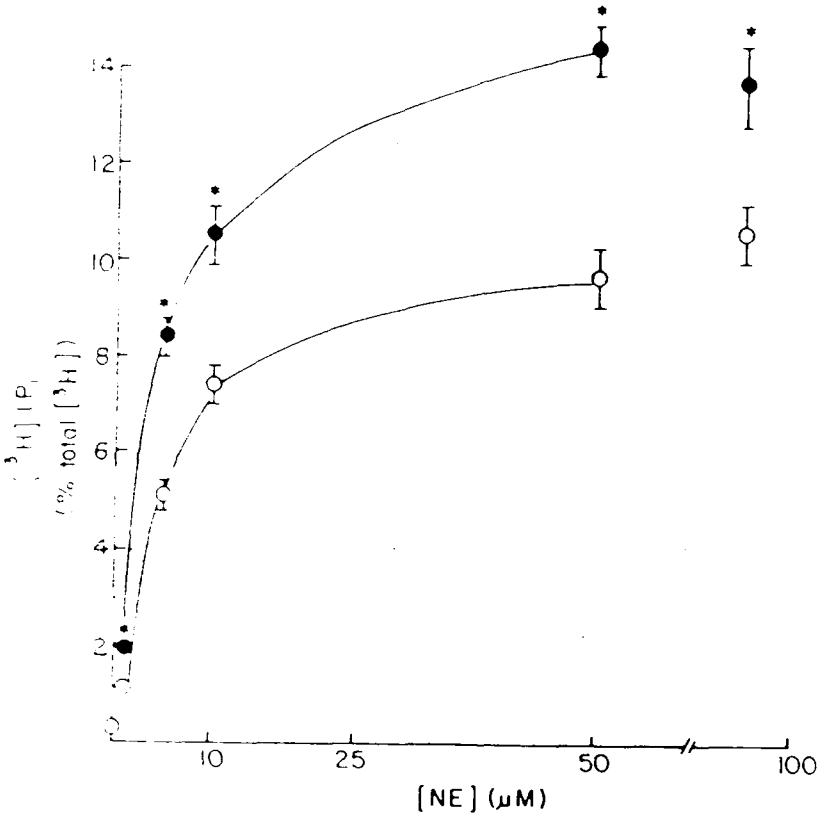


Figure 15. Effects of multiple modulators on NE-stimulated [^{3}H]IP$_1$ production.

Cerebral cortical slices were prelabelled with [^{3}H]inositol for 60 min followed by several washes. The labelled slices were incubated with no addition (basal), 300 μM baclofen (BACL), 200 μM NE, 500 μM quisqualate (QA), 200 μM arachidonic acid (AA), 300 μM GABA in the combinations indicated for 60 min and [^{3}H]IP$_1$ was measured as described in Methods. Values are given as [^{3}H]IP$_1$ produced as a percent of the total [^{3}H] in the slices, means ± SE of three experiments measured in triplicate. *$p < 0.05$ compared with NE alone. The response with NE, baclofen and AA was significantly ($p < 0.05$) greater than with NE and AA.

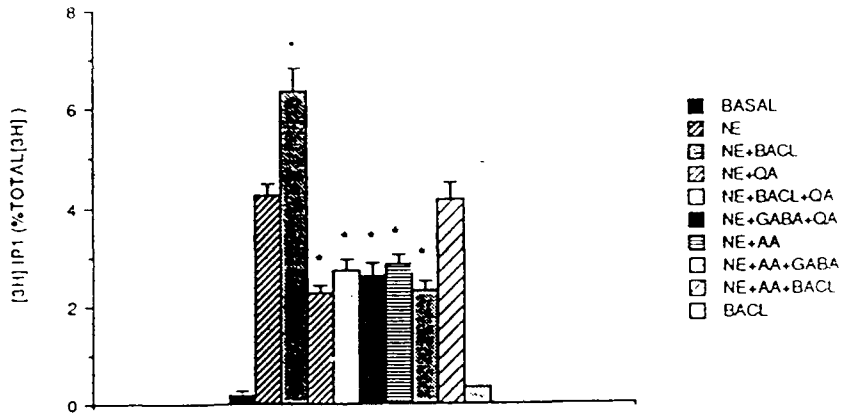


Figure 16. Effect of NMDA antagonists on phosphoinositide hydrolysis.

Prelabelled cortical slices were incubated (1 hr, 37°C) with 10^{-5}-2 x 10^{-3}M MK-801 (Δ), PCP (O) or AP-7 (●). The points represent ± SEM of three experiments measured in triplicate.

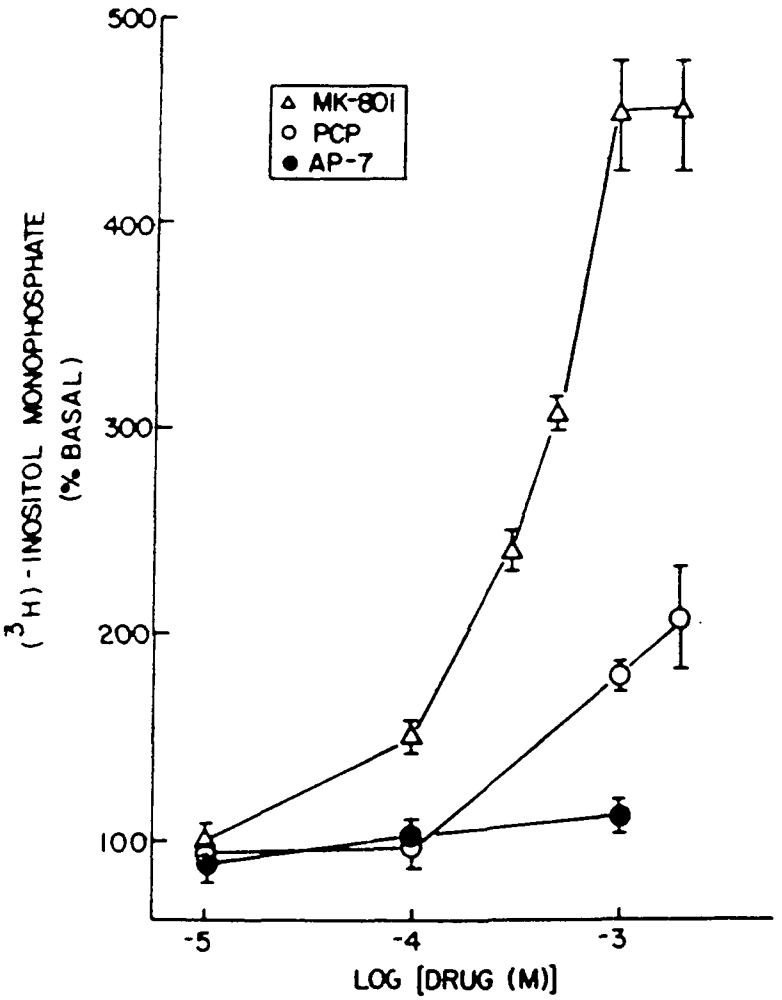


Figure 17. Effect of NMDA and glycine on MK-801-stimulated phosphoinositide hydrolysis.

Prelabelled cortical slices were incubated in the absence (open bars) or presence of MK-801 (500 μM, shaded bars) in the absence (control, CTL) or presence of glycine (0.1, 1.0 mM), NMDA (1 mM) or Zn^{2+} (500 μM). The assay was carried out in Mg^{2+}-containing (panel A) or Mg^{2+}-free (panel B) buffer. Glycine or Zn^{2+} alone did not affect (^3H)-inositol monophosphate accumulation (not shown). The bars represent mean ± SEM of three experiments measured in triplicate.

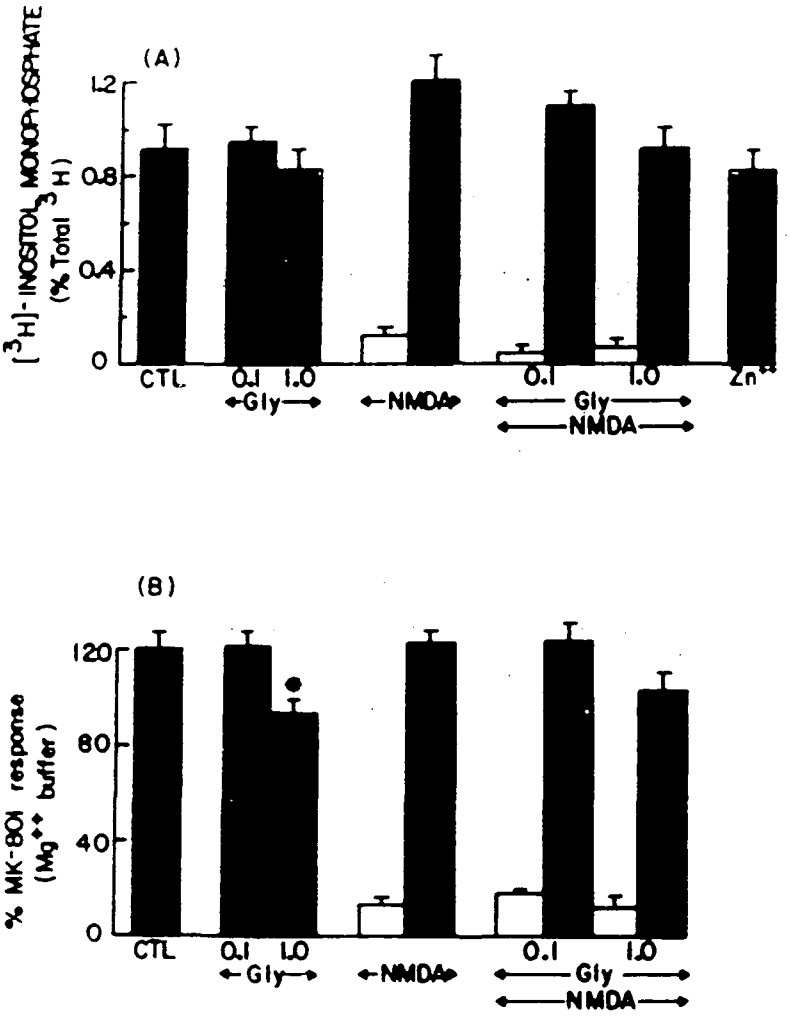


Figure 18. Na^+-dependence of $[^3H]IP_1$ production.

Cortical slices were prelabelled with [^{3}H]inositol for 60 min and washed, as described in the Methods. Slices were incubated for 60 min in media with the indicated concentrations of Na^+ in the absence (Basal) or presence of 100 μM norepinephrine (NE). Values are means ± S.E.M. from four experiments measured in triplicate. The inset shows the values for (*o*) basal and (●) norepinephrine-stimulated $[^3H]IP_1$ production after subtraction of the values obtained with 120 mM Na^+. These calculated values show that reduced Na^+ independently enhanced the response with each condition.

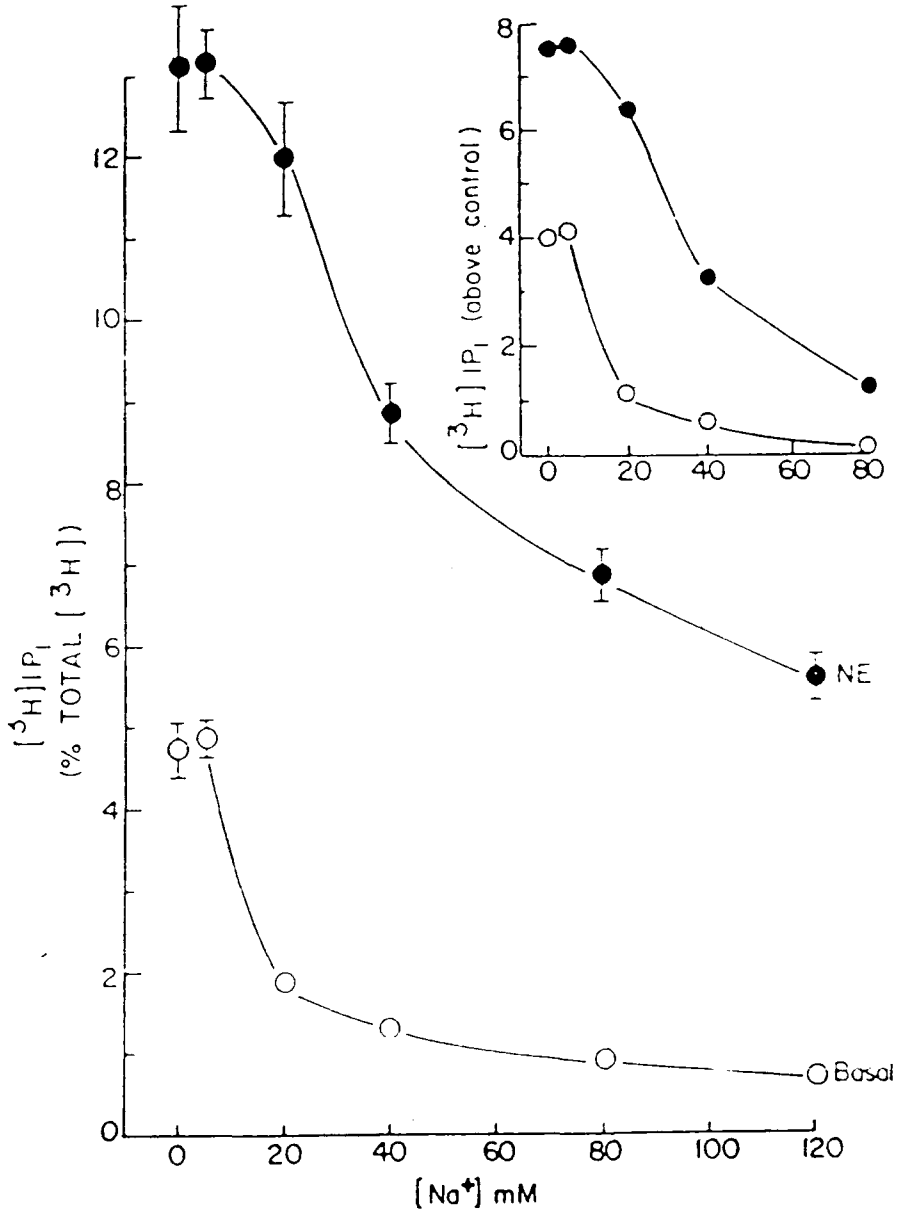


Figure 19. Effects of tetrodotoxin on [^{3}H]IP$_1$ production.

Cortical slices that had been prelabelled with [^{3}H]inositol and washed were incubated for 60 min with 100 μM norepinephrine (NE) (▲) or 2 mM carbachol (●) in the presence of varying concentrations of tetrodotoxin. Values are means ± S.E.M. from three experiments measured in triplicate. *$p < 0.05$ compared with no tetrodotoxin.

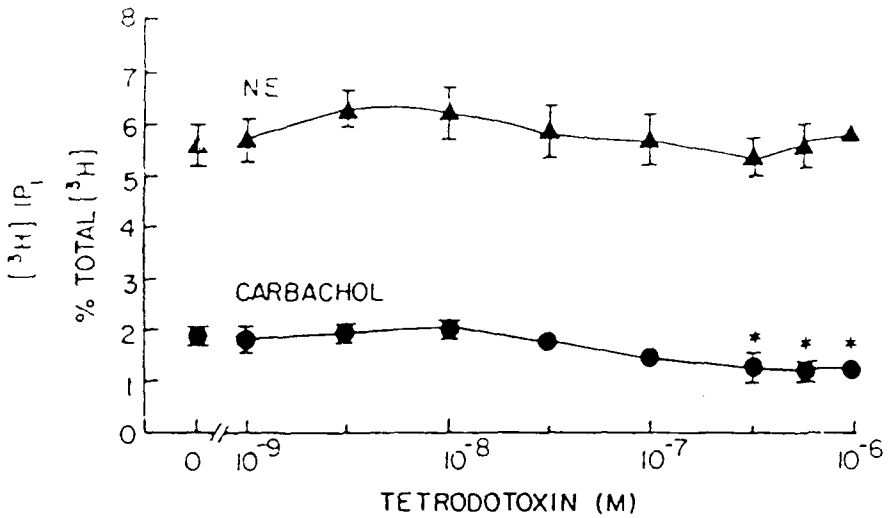


Figure 20. Effects of reduced Na^+ or tetrodotoxin on the modulation of phosphoinositide hydrolysis.

Cortical slices were prelabelled with [^{3}H]inositol for 60 min and washed, as described in the Methods. Slices were incubated for 60 min in normal media containing 120 mM Na^+ (control; open bars), media with 5 x 10^{-7} M tetrodotoxin (TTX; hatched bars), or media with 5 mM Na^+ (low Na^+; solid bars). The production of [^{3}H]IP_1 was measured after incubation with no added agonist (Basal), 100 μM norepinephrine (NE), 500 μM quisqualate (QA), NE plus QA, NE plus 500 μM glutamate (Glu), NE plus 200 μM arachidonic acid (AA), 2 mM carbachol (CARB), or 0.1 or 1.0 mM ibotenate (IBO). Values are means ± S.E.M. from three experiments (except n = 2 for IBO) measured in triplicate.

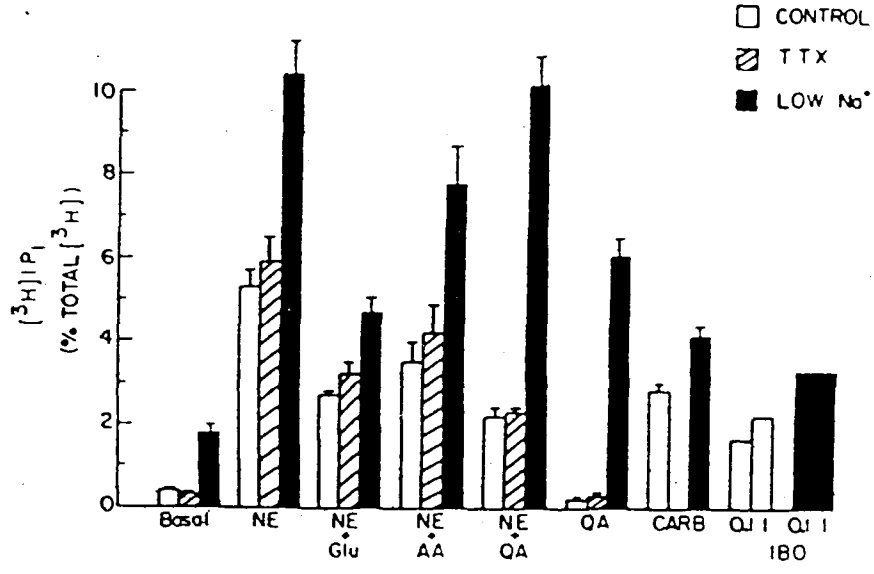


Figure 21. Low Na^+ blocked L-BOAA-induced inhibition of norepinephrine-stimulated $[^3H]IP_1$ production.

Cortical slices that had been prelabelled with $[^3H]$inositol and washed were incubated for 60 min with no addition (BSL), 100 μM norepinephrine (NE; hatched bars), 0.3 or 1 mM L-BOAA, or L-BOAA and NE (hatched bars), in media containing 5 mM Na^+ (Low Na^+) or 120 mM Na^+. (Normal Na^+). Values are means ± S.E.M. of three experiments measured in triplicate.

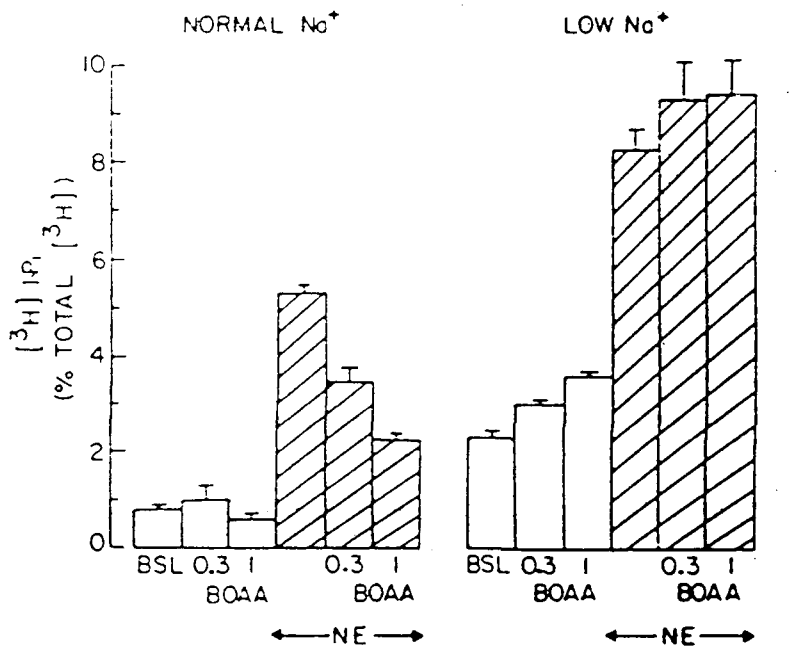


Figure 22. Effect of Na^+ on $[^3H]IP_1$ produced in response to quisqualate.

Cortical slices that had been prelabelled with [^{3}H]inositol and washed were incubated for 60 min with no agonist (BSL) or with concentrations of quisqualate (QA) varying from 10^{-7} to 10^{-3}M. Hatched bars indicate $[^3H]IP_1$ produced in response to QA after subtraction of the corresponding basal (BSL, left columns) value. Values are means ± S.E.M. of three experiments measured in triplicate.

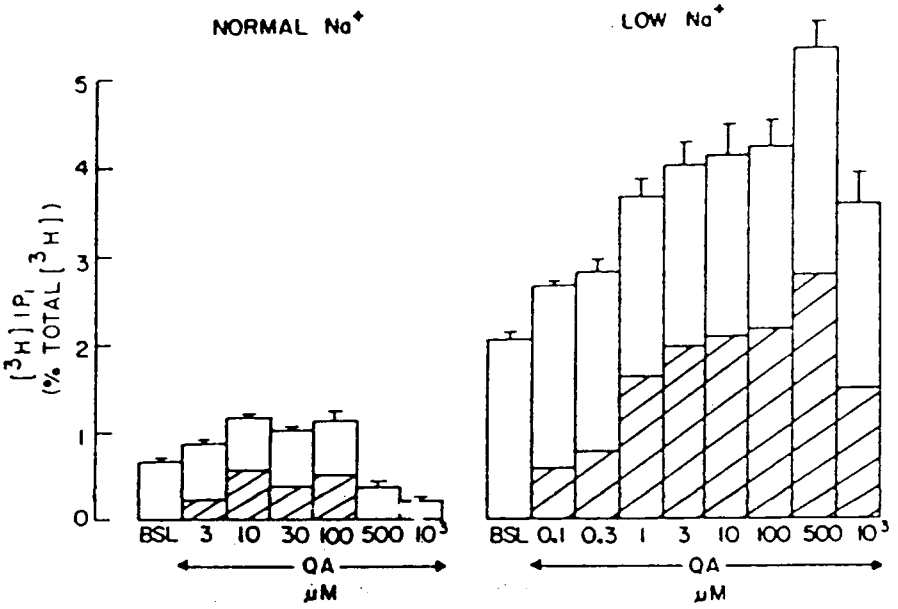


Figure 23. Effect of Na^+ on phosphoinositide hydrolysis in hippocampal slices.

Hippocampal slices that had been prelabelled with [^{3}H]inositol and washed were incubated for 60 min in the absence of added agonist (basal, B, open bars), with 100 μM quisqualate (Q, hatched bars), or with 100 μM norepinephrine (N, solid bars) in medium containing 120 mM Na^+ (Normal Na^+) or 5 mM Na^+ (Low Na^+). [^{3}H]IP_1 (left ordinate), [^{3}H]IP_2, and [^{3}H]IP_3 (right ordinate) were measured as described in the Methods. Values are means ± S.E.M. of three experiments measured in triplicate.

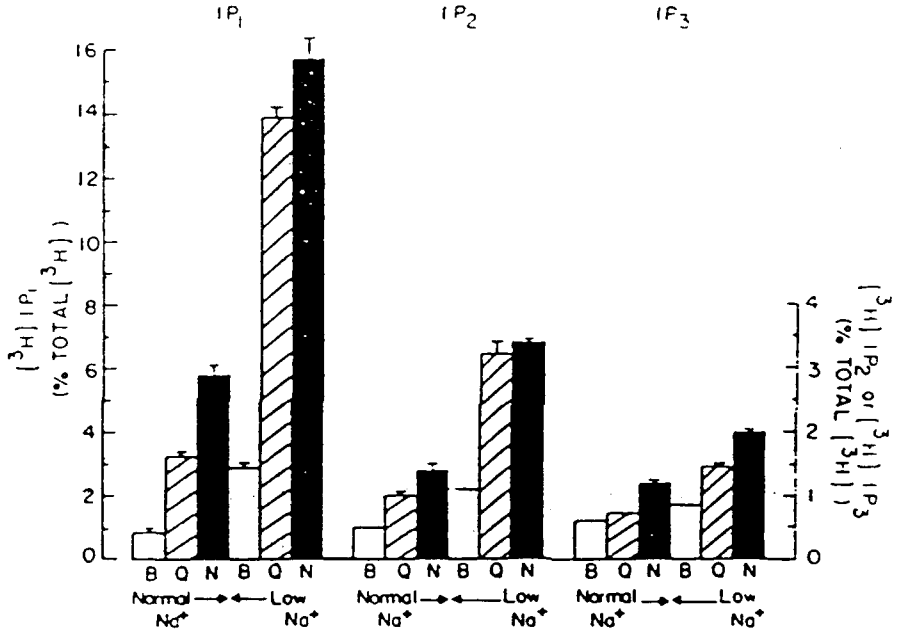


Figure 24. Basal, calcium-dependent, and protein kinase C-dependent protein phosphorylation in hippocampal particulate fractions of high (A) and low (B) molecular mass proteins from control (-) and chronic lithium-treated (+) rats. Assays and SDS-PAGE were conducted as described in the Methods, using 6.5% gels to separate proteins > 45 kD and 12% gels to separate proteins < 45 kD. Autoradiographs show representative results from one control and one lithium-treated rat: (A) phosphorylation measured under basal conditions (lanes 1 and 2), in the presence of 1.5 mM $CaCl_2$ (lanes 3-6), or in the presence of $CaCl_2$, 10 µg PS, 1 µM PMA (lanes 7 and 8); and (B) phosphorylation in the presence of $CaCl_2$ (lanes 1 and 2) or in the presence of $CaCl_2$, 10 µg PS, and 1 µM PMA (lanes 3 and 4). Apparent molecular masses (kD) of major phosphoproteins are indicated.

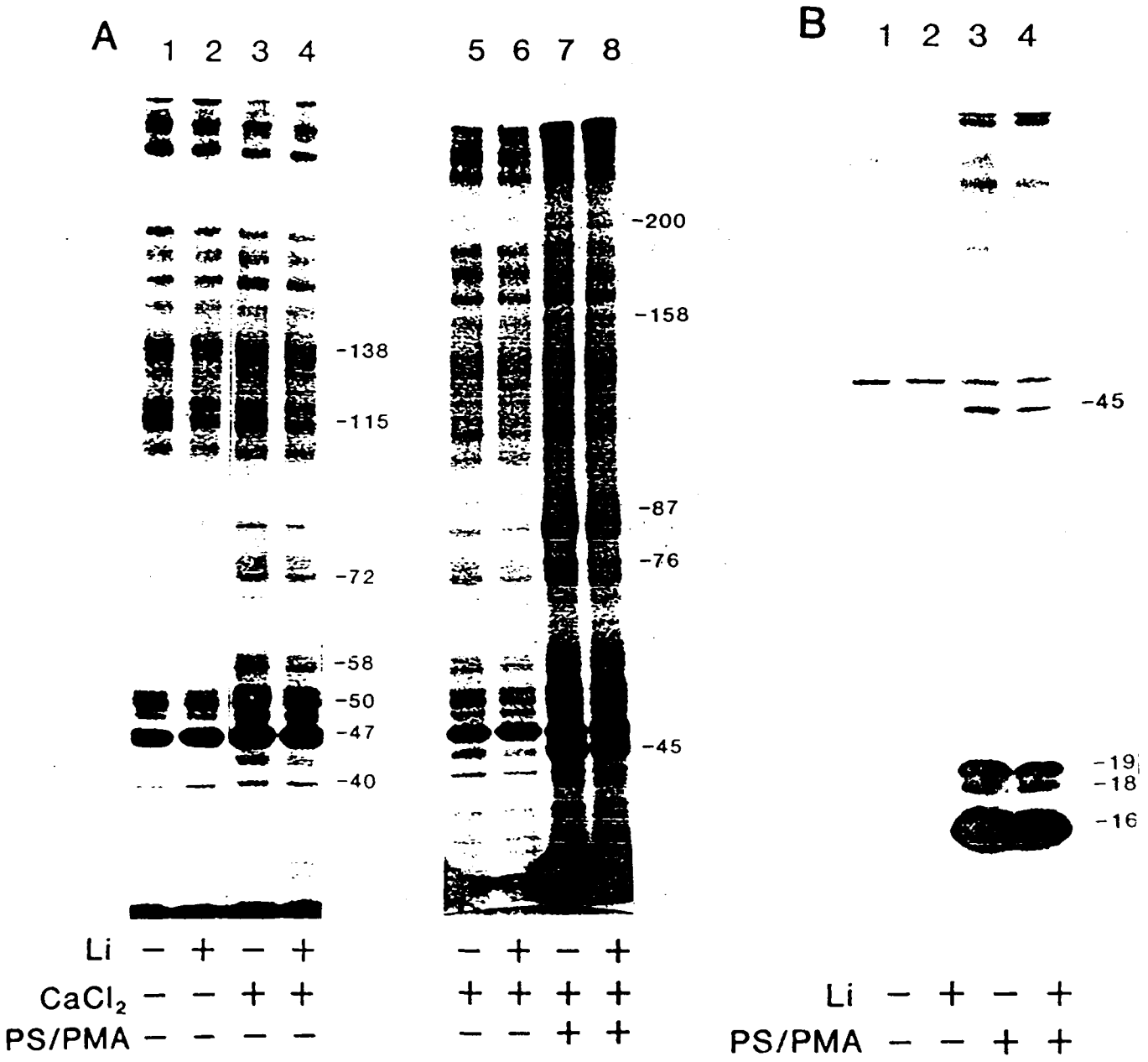


Figure 25. Cyclic AMP-dependent phosphorylation of hippocampal particulate proteins from control (-) and chronic lithium-treated (+) rats. Particulate proteins were phosphorylated as described in the Methods and separated by SDS-PAGE on 6.5% gels. Phosphorylation was measured in the presence of 1 mM theophylline (lanes 1 and 2) or in the presence of theophylline and 50 μM cAMP (lanes 3 and 4). Apparent molecular masses (kD) of major phosphoproteins are indicated.

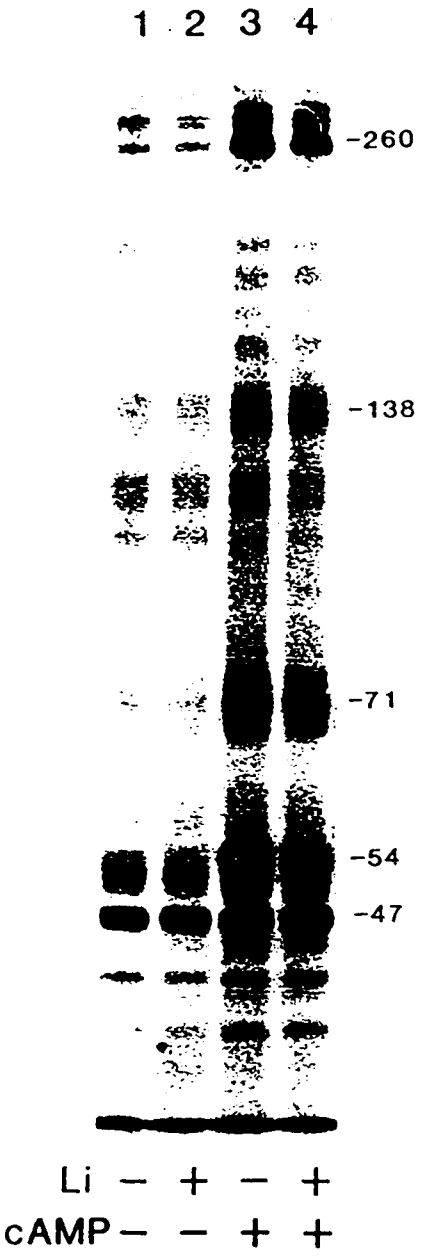


Figure 26. Calcium-dependent and protein kinase C-dependent protein phosphorylation in hippocampal soluble fractions of high (A) and low (B) molecular mass proteins from control (-) and chronic lithium-treated (+) rats. Assays and SDS-PAGE were conducted as described in the Methods, using 6.5% gels to separate proteins > 45 kD and 12% gels to separate proteins < 45 kD. Phosphorylation was measured in the presence of 1.5 mM $CaCl_2$ (lanes 1 and 2), or in the presence of $CaCl_2$, 10 μg PS, and 1 μM PMA (lanes 3 and 4). Apparent molecular masses (kD) of major phosphoproteins are indicated.

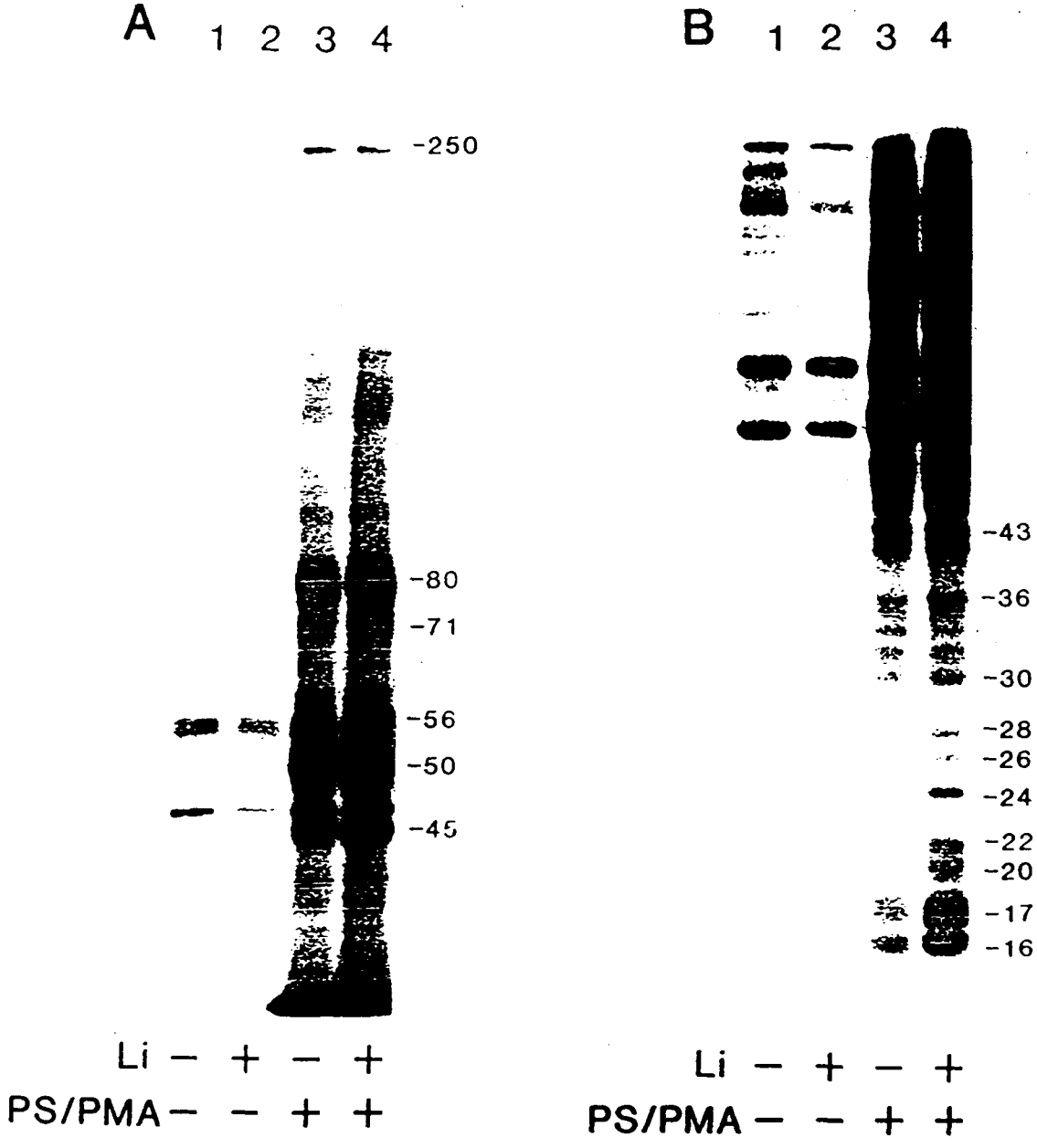


Figure 27. Phosphoinositide hydrolysis in hippocampus.

Rats were treated with LiCl (L; 3 mmole/kg; ip; 20 hr prior) or LiCl plus pilocarpine (LP; 30 mg/kg; sc; 25 or 90 min prior) and then hippocampal slices were prepared and prelabelled with [^{3}H]inositol as described in the Methods. Slices were incubated with 1 mM ibotenate (Ibo), 2 mM carbachol (Carb), 2 mM carbachol plus 15 mM KCl, 100 µM norepinephrine (NE), or 55 mM KCl, followed by measurement of [^{3}H]inositol monophosphate [^{3}H]IP$_1$). Values are means ± SEM and are given as a percent of total [^{3}H] incorporated into the slices. n = 3-4, *p < 0.05 compared with controls.

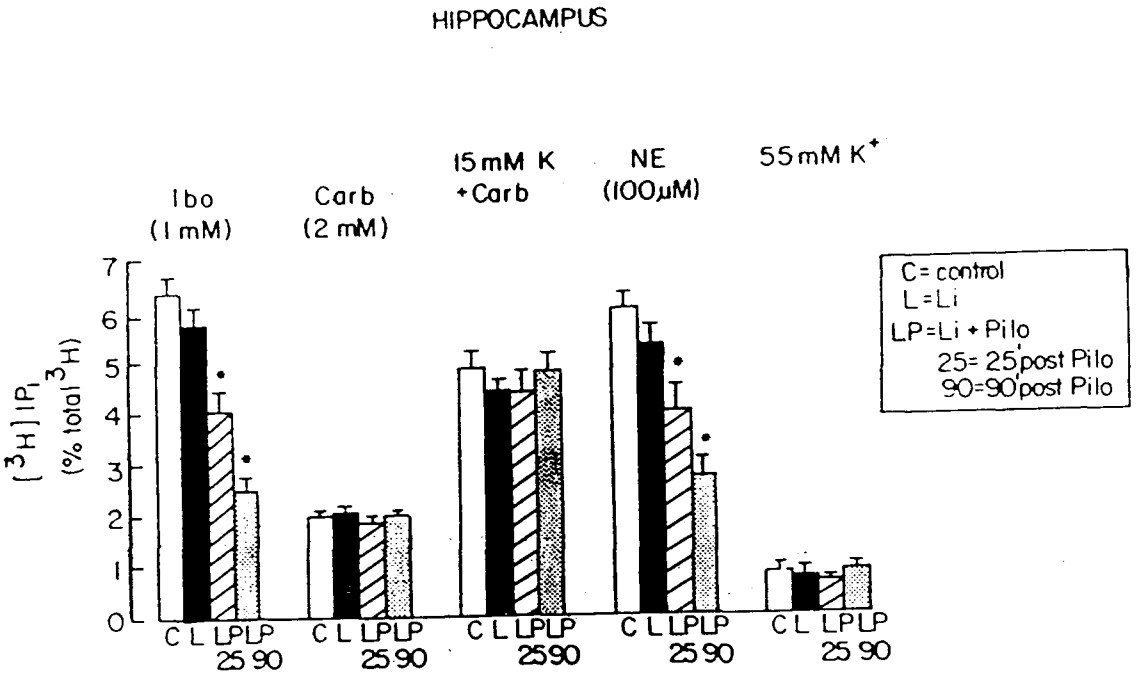


Figure 28. Norepinephrine-stimulated phosphoinositide hydrolysis in hippocampus.

Rats were treated as described in the legend to Figure 27 followed by measurement of [^{3}H]inositol monophosphate production in the presence of 10^{-6} to 10^{-4} M norepinephrine (NE) as described in the Methods. All values from rats treated with LiCl plus pilocarpine (Li + Pilo) at concentrations of 3 x 10^{-6} M NE and greater were significantly less than controls, ($p < 0.05$), n = 3.

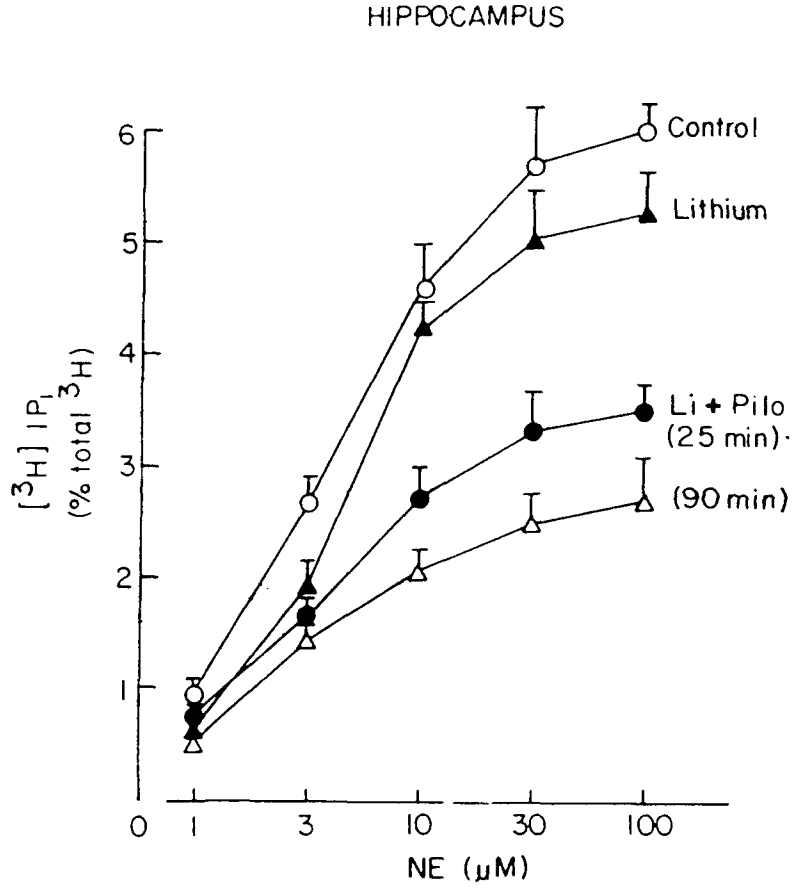


Figure 29. Phosphoinositide hydrolysis in cortical slices.

Cortical slices prepared from control (C) or treated (S; seizing) rats (LiCl plus pilocarpine; 60 min) were prelabelled with [^{3}H]inositol as described in the Methods. Slices were incubated in normal buffer containing 120 mM Na^+ or in buffer containing 5 mM Na^+ (NaCl replaced with choline chloride) and with 2 mM carbachol (CARB), 100 μM norepinephrine (NE), 100 μM quisqualate (QA), or 100 μM NE plus 500 μM QA. n = 3.

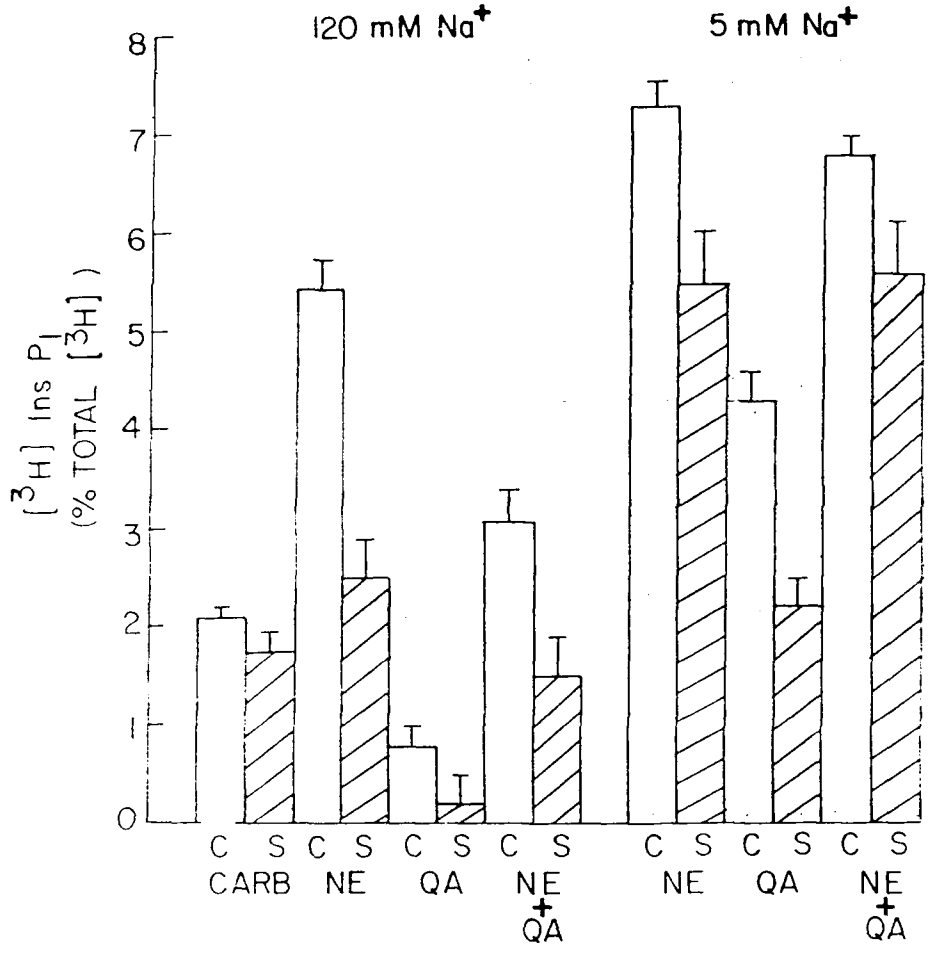


Figure 30. NaF-stimulated phosphoinositide hydrolysis.

Cortical slices prepared from control or treated (seizing) rats (LiCl plus pilocarpine; 60 min) were prelabelled with [^{3}H]inositol as described in the Methods. Slices were incubated with the indicated concentration of NaF in the absence of Ca^{2+} (which enhances the response to NaF) followed by measurement of [^{3}H]inositol monophosphate ([^{3}H]InsP_1). n = 3

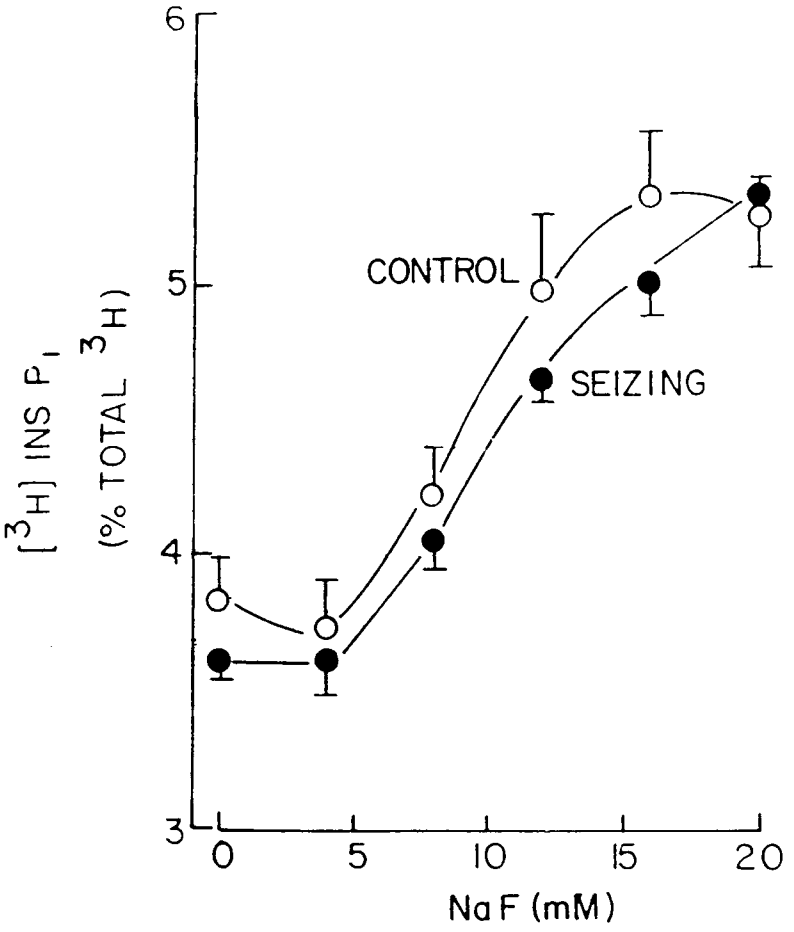


Figure 31. *Inhibition of phosphoinositide hydrolysis by activation of protein kinase C by PDBu.*

Rats were treated with lithium plus pilocarpine (60 min prior) and phosphoinositide hydrolysis was measured as described in the Methods in cerebral cortical slices incubated with 2 mM carbachol (CARB), 100 μM norepinephrine (NE), or NE plus 1 mM quisqualate (NE + QA) in the absence (open bars) or presence (hatched bars) of 1 μM PDBu. Values are means ± SEM (n=4) and are given as a percent of the total [^{3}H] incorporated into the slices. *p < 0.05 compared with values obtained in the absence of PDBu.

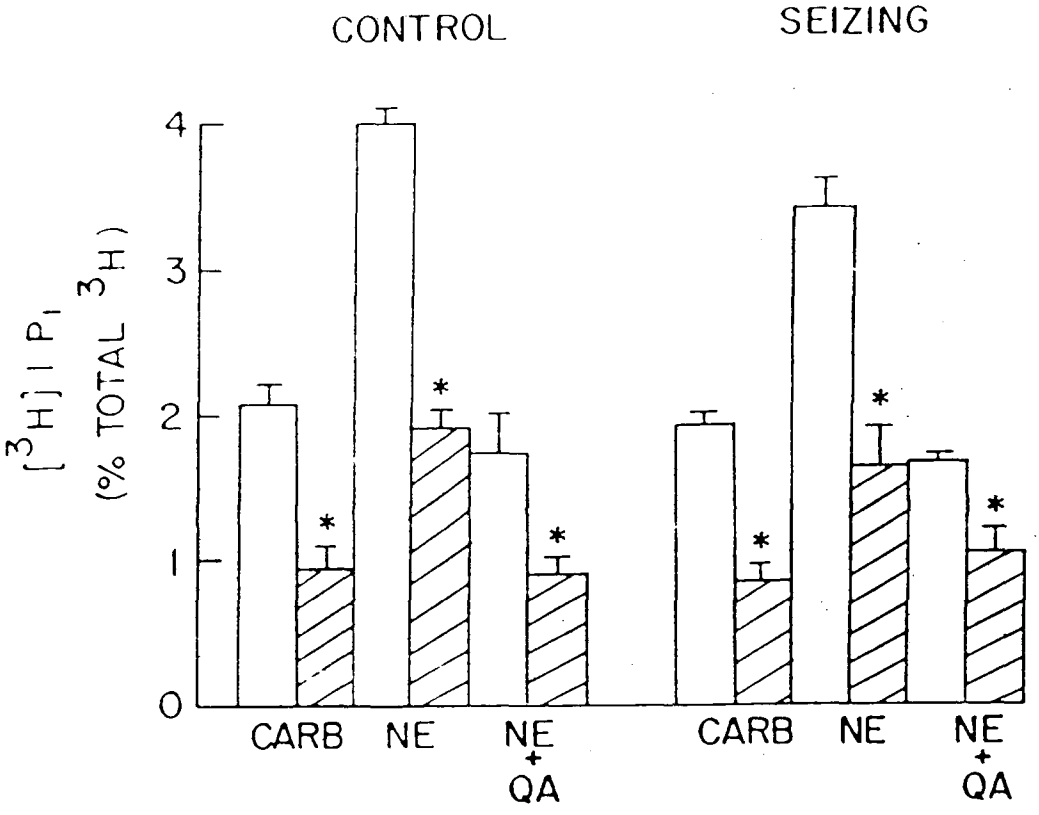


Figure 32. Phosphoinositide hydrolysis after DFP treatment.

Rats were treated with DFP (2.8 mg/kg; sc; 20 min prior) followed by measurement of [^{3}H]inositol monophosphate ([^{3}H]IP$_1$) production in hippocampal (open bars) and cortical (hatched bars) slices after incubation with 2 mM carbachol (CARB), 2 mM carbachol plus 15 mM KCl (CARB, K) or 100 μM norepinephrine (NE) as described in the Methods. Values are means ± SEM and are given as a percent of the total [^{3}H]' incorporated into the slices. n = 3 for each group. *p < 0.05 compared with controls.

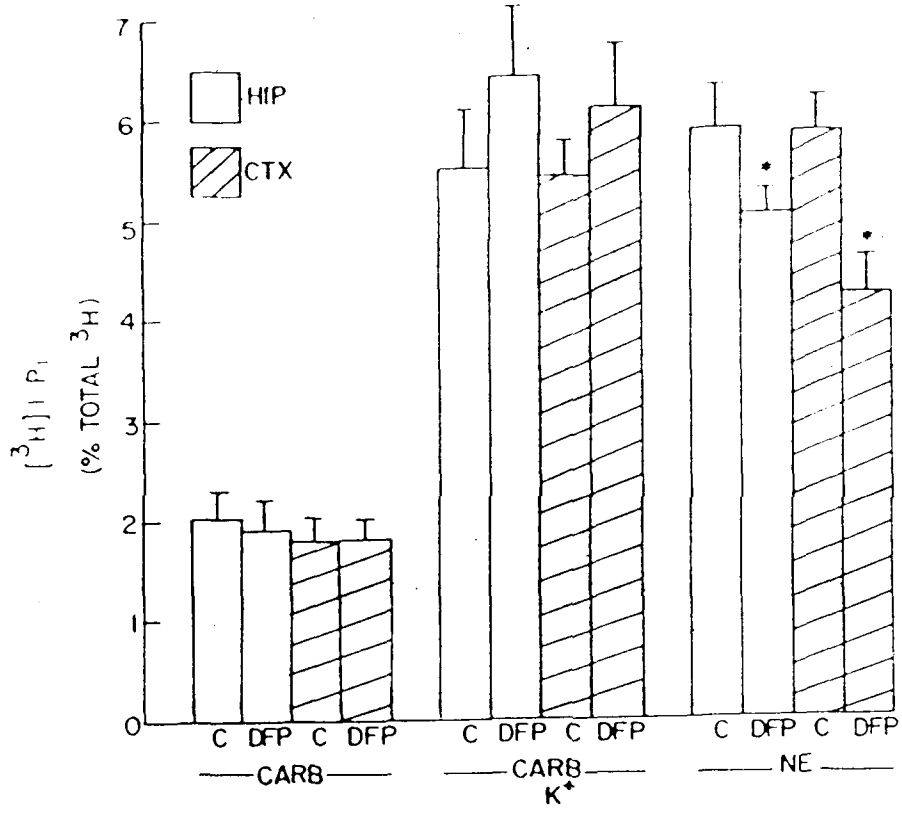


Figure 33. Protein kinase C activity in rat cerebral cortex.

The activity of protein kinase C was measured in the cytosolic (top) or membrane (middle) fraction prepared from rat cerebral cortex. Rats were treated with (i) the combination of LiCl (3 mmole/kg; ip; 20 hr prior) plus pilocarpine (30 mg/kg; sc; 10, 20, 60 or 120 min prior), (ii) LiCl alone, (iii) kainate (10 mg/kg; sc; 20 or 96 min prior), or LiCl plus kainate. The bottom panel shows the % of total protein kinase C activity associated with the membrane fraction.

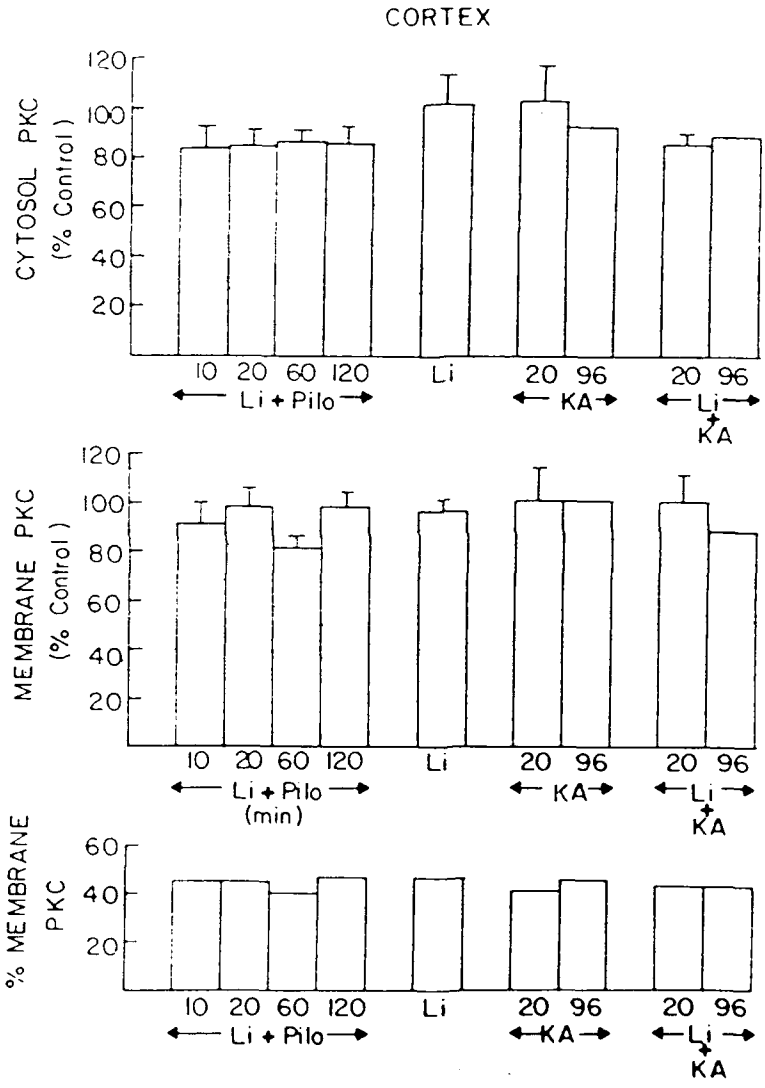


Figure 34. Protein kinase C activity in rat hippocampus.

The activity of protein kinase C was measured in the cytosolic (top) or membrane (middle) fraction prepared from the hippocampus of rats treated as described in the legend to Figure 33. The bottom panel shows the % of total protein kinase C activity associated with the membrane fraction.

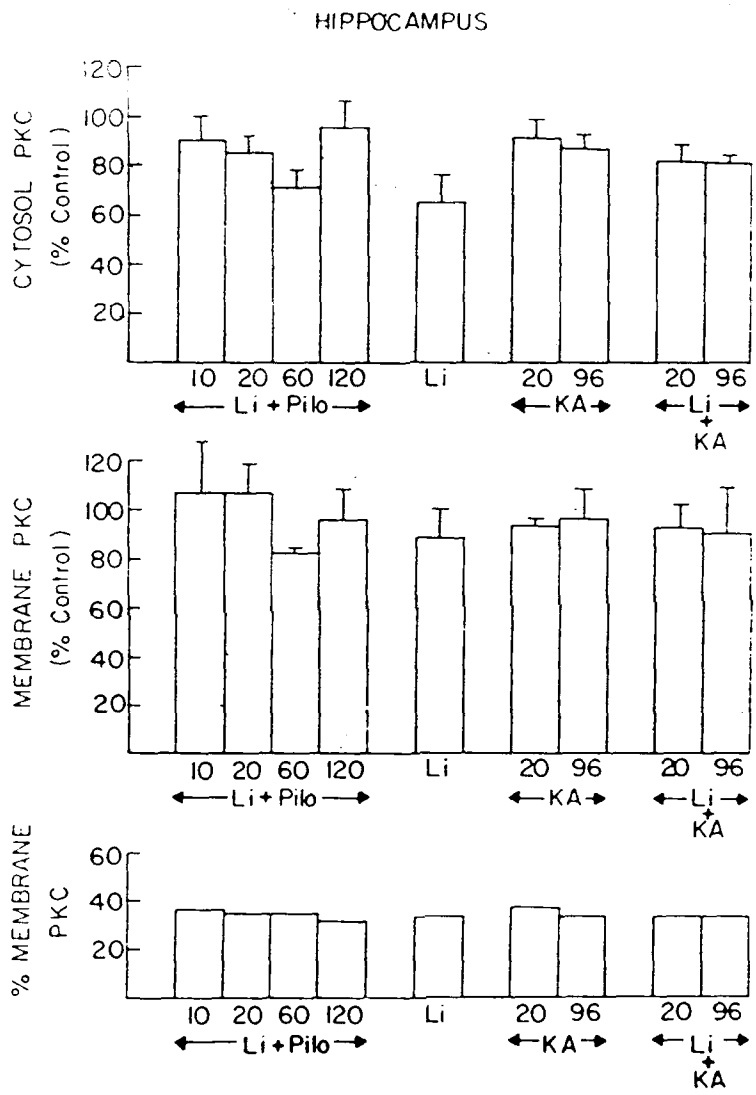


Figure 35. Immunoblots of phosphotyrosine proteins in rat cortex and hippocampus after sacrifice by decapitation (D) or focussed-beam microwave irradiation (M). Molecular masses (kD) of phosphotyrosine proteins analyzed quantitatively are indicated.

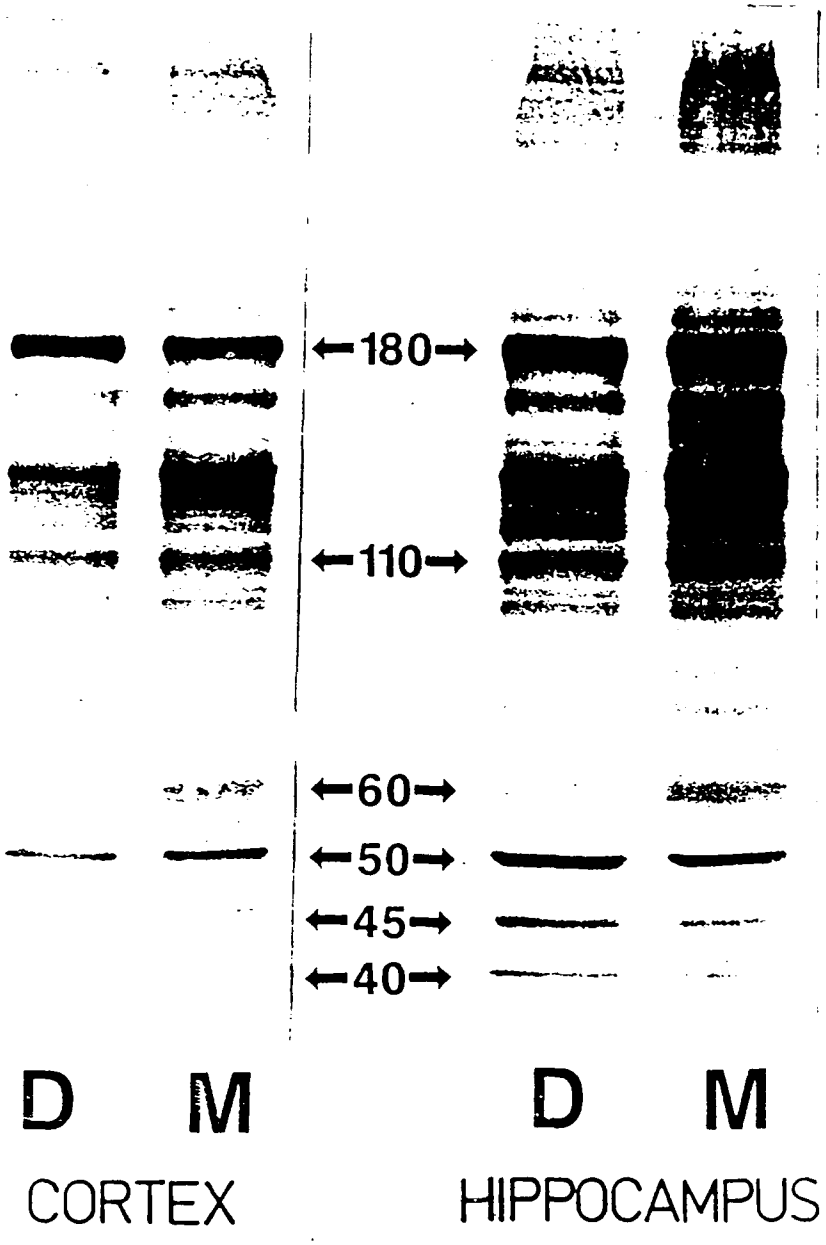


Figure 36. Immunoblots of phosphotyrosine proteins in rat brain regions.

Saline-treated controls (-) or rats in which seizures were induced (+) by administration of LiCl (3 mmole/kg, ip, 20 hr prior) and pilocarpine (30 mg/kg, sc, 1 hr prior) were sacrificed by focussed-beam microwave irradiation and phosphotyrosine proteins were identified as described in the Methods, in the cerebral cortex (CTX), hippocampus (HIP), and striatum (STM). Molecular masses (kD) are indicated for the phosphotyrosine proteins which were analyzed quantitatively in further experiments.

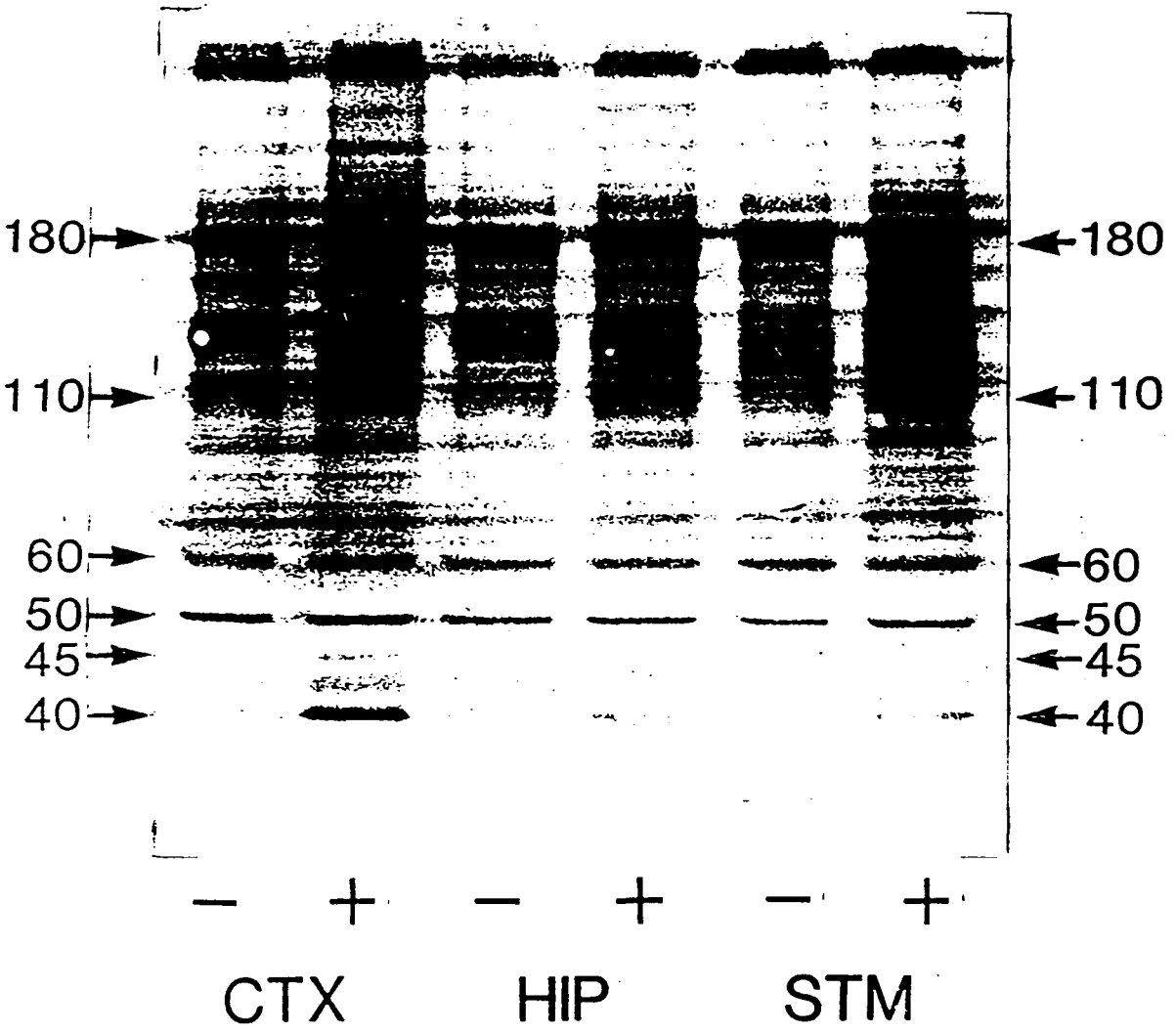


Figure 37. Quantitative analysis of hippocampal phosphotyrosine proteins during initiation and maintenance of status epilepticus.

Rats were treated with LiCl (3 mmol/kg, ip, 20 hr prior) and pilocarpine (30 mg/kg, sc) and sacrificed by focussed-beam microwave irradiation after 10 min (before paroxysmal spikes), 20 min (when spike trains are observed by EEG analysis), 25 min (immediately after the first tonic-clonic seizure signalling the initiation of status epilepticus), 60 min (during status epilepticus) and 120 min (during status epilepticus). Values were compared with controls run in parallel and are means ± SEM of 3-4 experiments.

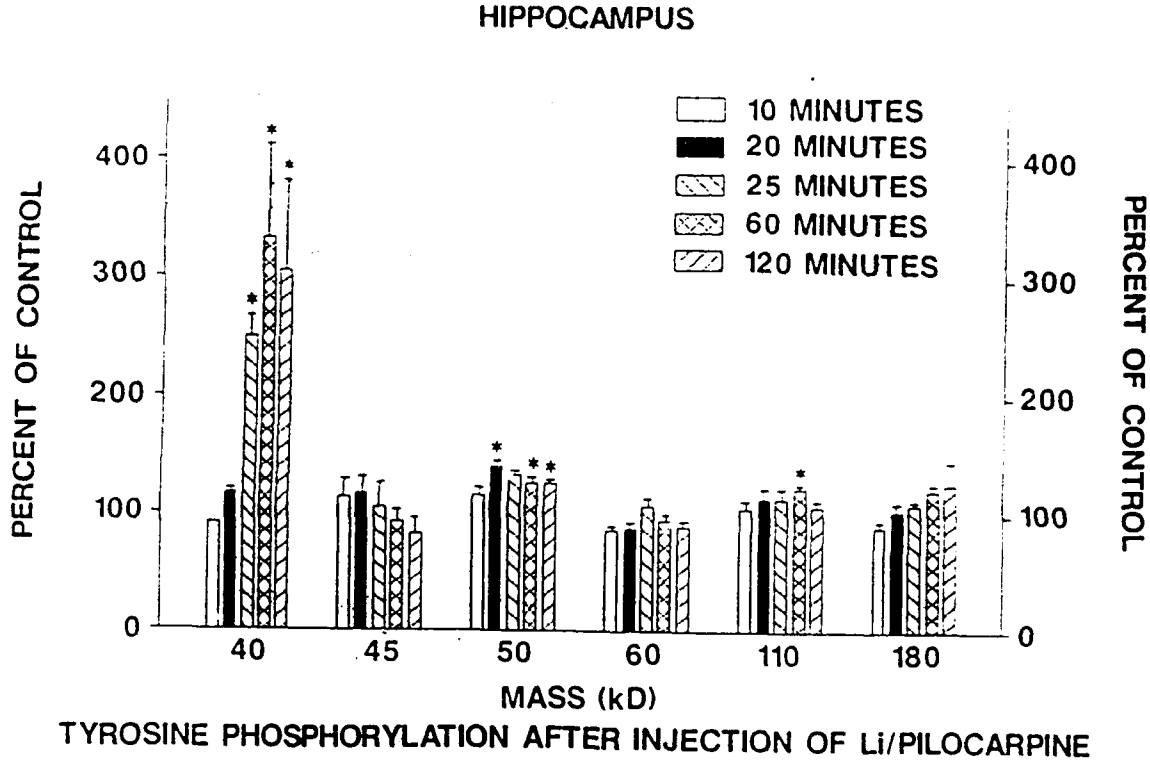


Figure 38. Quantitative analysis of phosphotyrosine proteins in rat cortex (A) and hippocampus (B).

Rats were (i) treated with lithium for 4 weeks (chronic Li), (ii) treated with pilocarpine (30 mg/kg, sc, 50 min), or (iii) treated with both chronic lithium and pilocarpine, which resulted in status epilepticus 15-20 min after pilocarpine administration. Rats were sacrificed by focussed-beam microwave irradiation 60 min after pilocarpine administration and the phosphotyrosine proteins were identified as described in the Methods. Values were compared with controls run in parallel and are means ± SEM of 3-4 experiments.

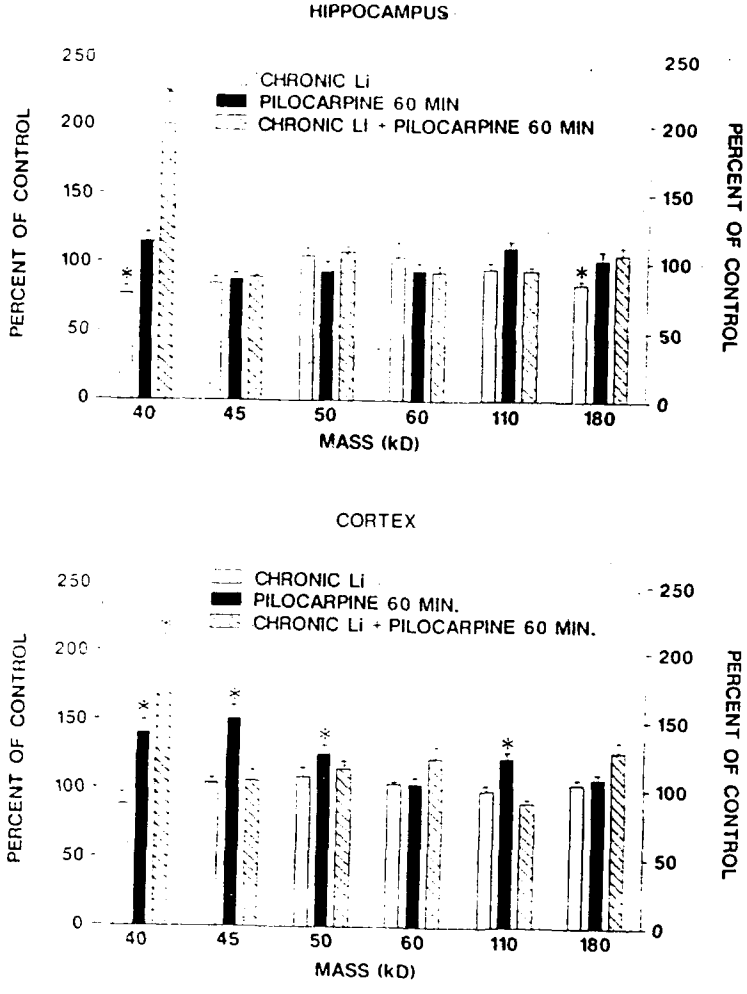


Figure 39. Quantitative analysis of phosphotyrosine proteins in rat hippocampus and cortex during kainate-induced seizures.

Rats were treated with kainate (10 mg/kg, sc), which resulted in status epilepticus in approximately 90 min, and were sacrificed by microwave irradiation 120 min after kainate administration. Values were compared with controls run in parallel and are means ± SEM of 3 experiments.

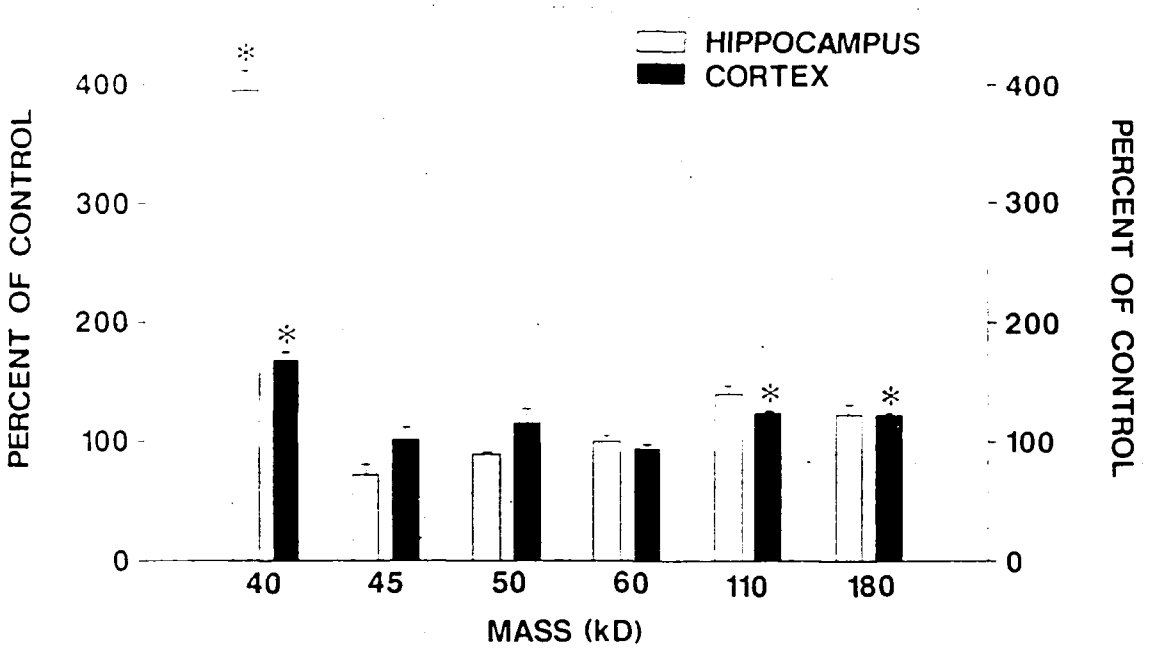


Figure 40. Subcellular localization of phosphotyrosine proteins.

Control (-) or seizing (+) rats (acute LiCl, 3 mmole/kg, ip, 20 hr prior, plus pilocarpine, 30 mg/kg, 60 min prior to sacrifice) were decapitated and cytosolic and membrane fractions from the cerebral cortex were analyzed for phosphotyrosine proteins.

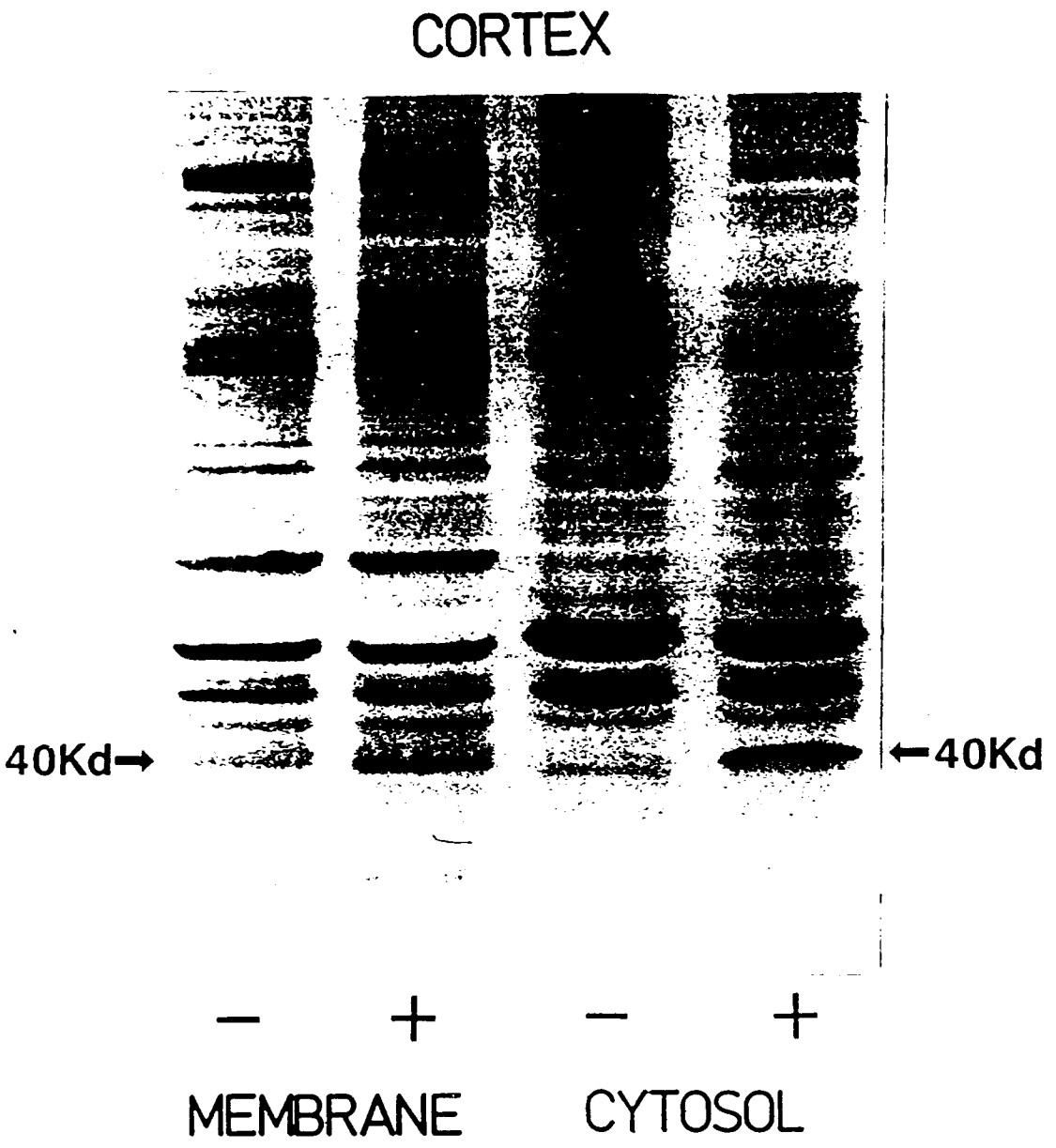


Figure 41. Effects of in vitro 8 mM lithium on [^{3}H]PI hydrolysis in cortical membranes from (A) control or (B) chronic lithium-treated rats.

Cortical membranes were incubated for 20 min at 37°C with [^{3}H]PI and no addition (BASAL), 1 mM carbachol (CARB), 3 μM GTPγS, carbachol plus GTPγS, 1 mM pilocarpine (PILO) plus GTPγS, or 20 mM NaF plus 10 μM $AlCl_3$, without added LiCl (open bars) or with 8 mM LiCl (hatched bars). Means ± SEM (n=3 rats for each group).

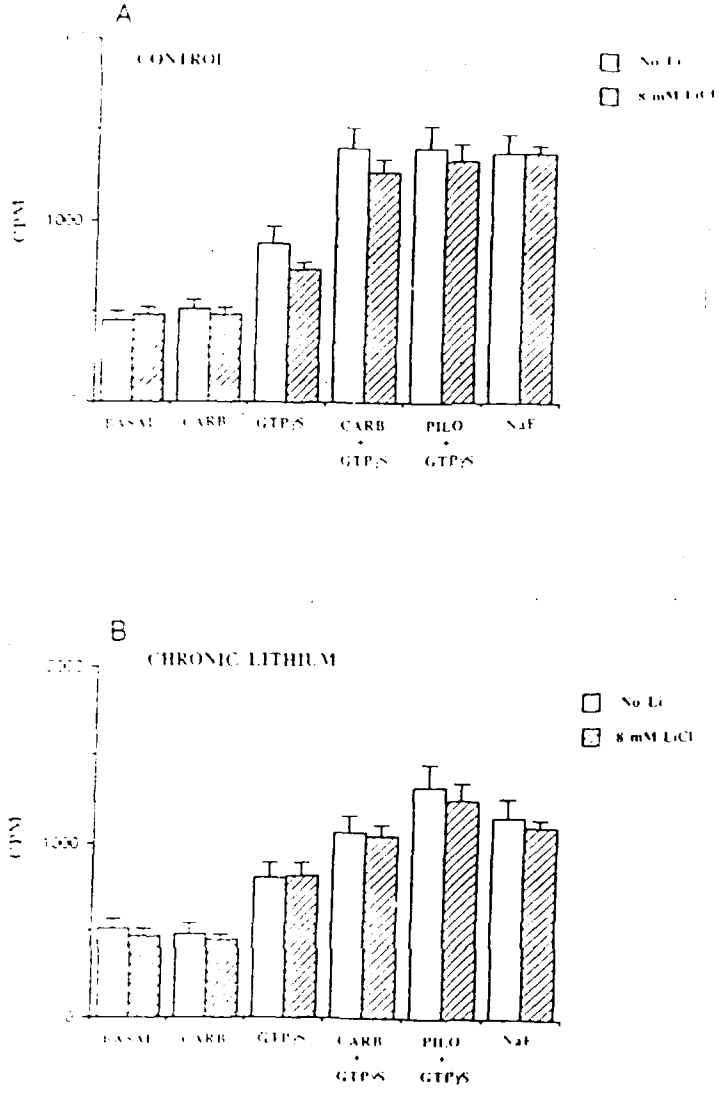


Figure 42. Concentration-response of [^{3}H]PI hydrolysis to (A) GTPγS alone or with carbachol and (B) NaF in hippocampal membranes from control or chronic lithium-treated rats.

Hippocampal membranes were incubated with [^{3}H]PI for 20 min at 37° with GTPγS (□ control; ■ chronic lithium-treated), GTPγS plus 1 mM carbachol (C control; ● chronic lithium treated), or NaF plus 10 μM $AlCl_3$ (△ control; ▲ chronic lithium-treated). Means ± SEM for 4 rats in each group. ÷ $p < 0.05$, * $p < 0.01$ compared with controls.

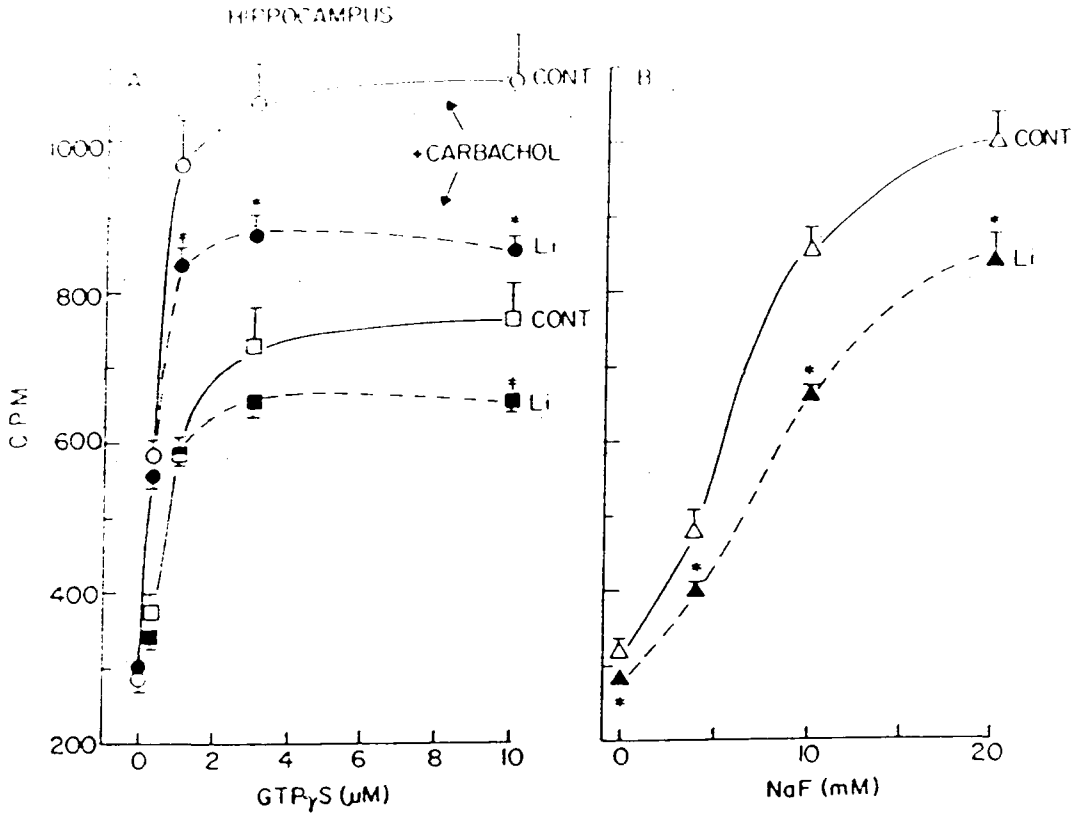


Figure 43. Concentration-response of [^{3}H]PI hydrolysis to (A) GTPγS alone or with carbachol and (B) NaF in striatal membranes from control or chronic lithium-treated rats.

Striatal membranes were incubated with [^{3}H]PI for 20 min at 37° with GTPγS (□ control; ■ chronic lithium treated), GTPγS plus 1 mM carbachol (○ control; ● chronic lithium treated), or NaF plus 10 μM $AlCl_3$ (△ control; ▲ chronic lithium-treated). Means ± SEM for 4 rats in each group. ‡ $p < 0.05$, * $p < 0.01$ compared with controls.

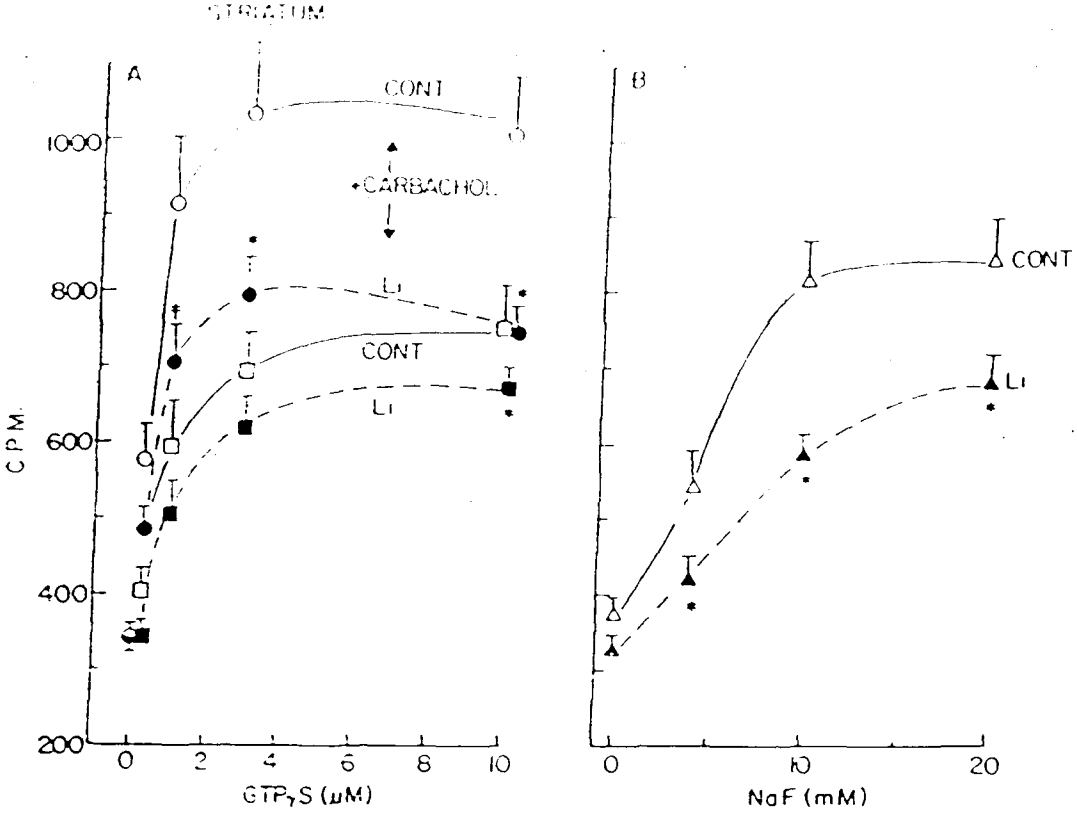


Figure 44. Concentration-response of [^{3}H]PI hydrolysis to (A) GTPγS alone or with carbachol and (B) NaF in cortical membranes from control or chronic lithium-treated rats.

Cortical membranes were incubated with [^{3}H]PI for 20 min at 37° with GTPγS (□ control; ■ chronic lithium treated), GTPγS plus 1 mM carbachol (○ control; ● chronic lithium treated), or NaF plus 10 μM $AlCl_3$ (△ control; ▲ chronic lithium-treated). Means ± SEM for 4 rats in each group. * $p < 0.01$ compared with controls.

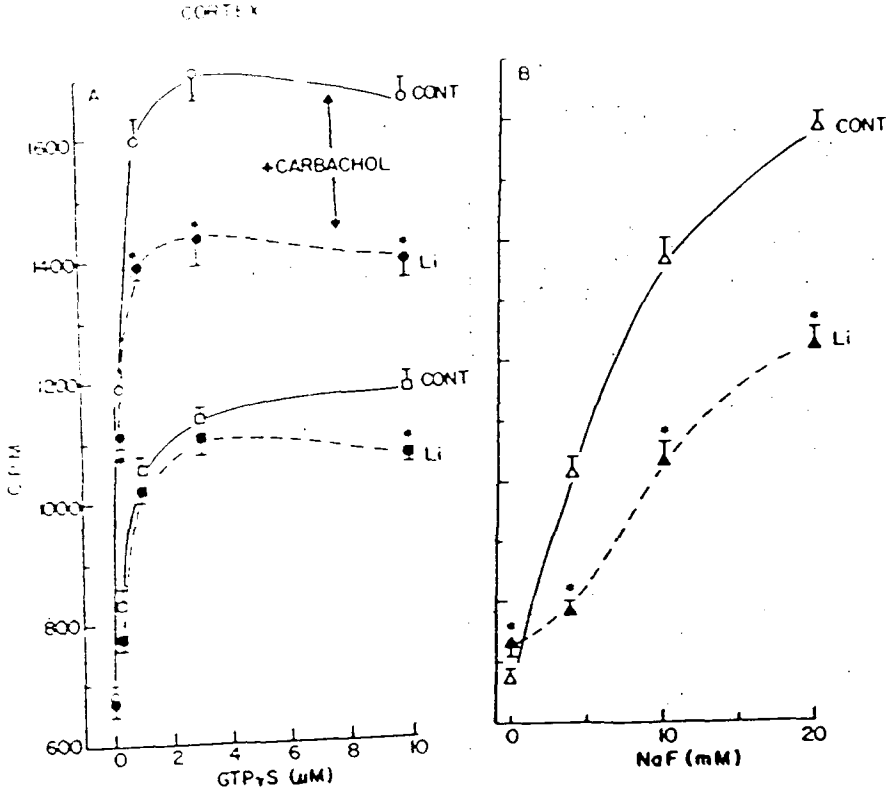


Figure 45. Agonist stimulated [^{3}H]PI hydrolysis in control hippocampal membranes.

Hippocampal membranes were incubated for 20 min at 37° with [^{3}H]PI and no agonist (BASAL), 1 mM quisqualate (QA), 1 mM trans-ACPD, 1 mM kainate (KA), 1 mM NMDA, 1 mM glutamate (GLUT) or 1 mM carbachol (CARB), each in the absence (open bars) or presence (filled bars) of 3 μM GTPγS. Means ± SEM (n = 3 different membrane preparations).

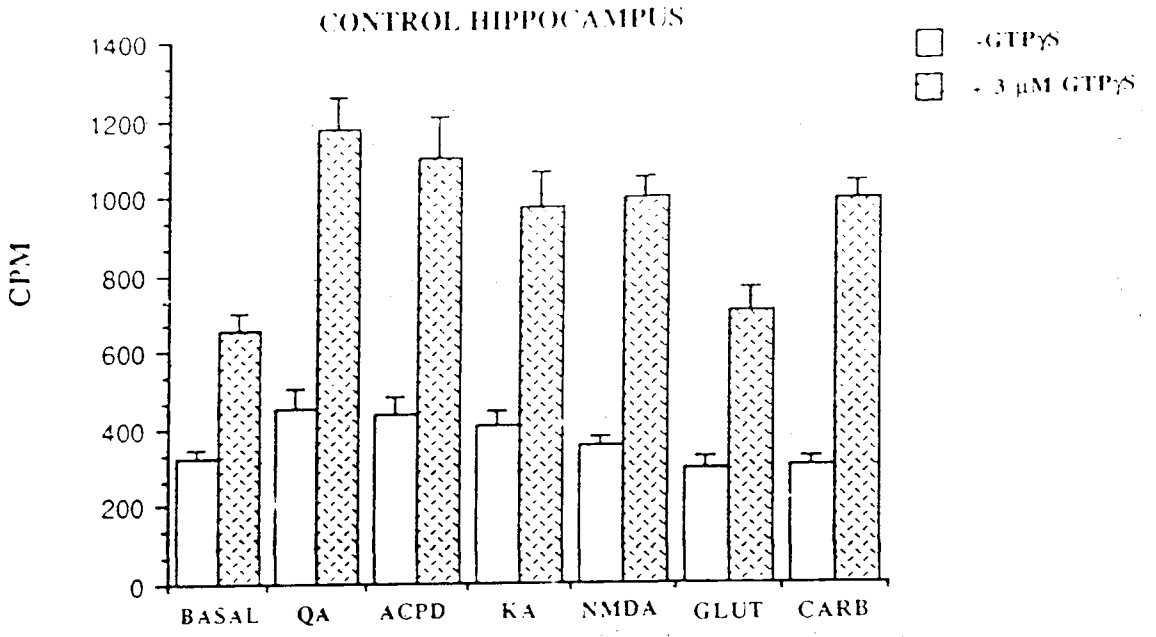


Figure 46. Effects of chronic lithium treatment on [^{3}H]PI hydrolysis in hippocampal membranes.

Hippocampal membranes were incubated for 20 min at 37°with [^{3}H]PI and no agonist (BASAL), 20 mM NaF plus 10 μM $AlCl_3$ (NAF), 1 mM quisqualate (QA), 3 μM GTPγS, or GTPγS plus quisqualate. Means ± SEM for membranes from 4 control (open bars) or 4 lithium-treated (filled bars) rats (different groups from those used in previous Figures). * $p < 0.01$ compared with controls.

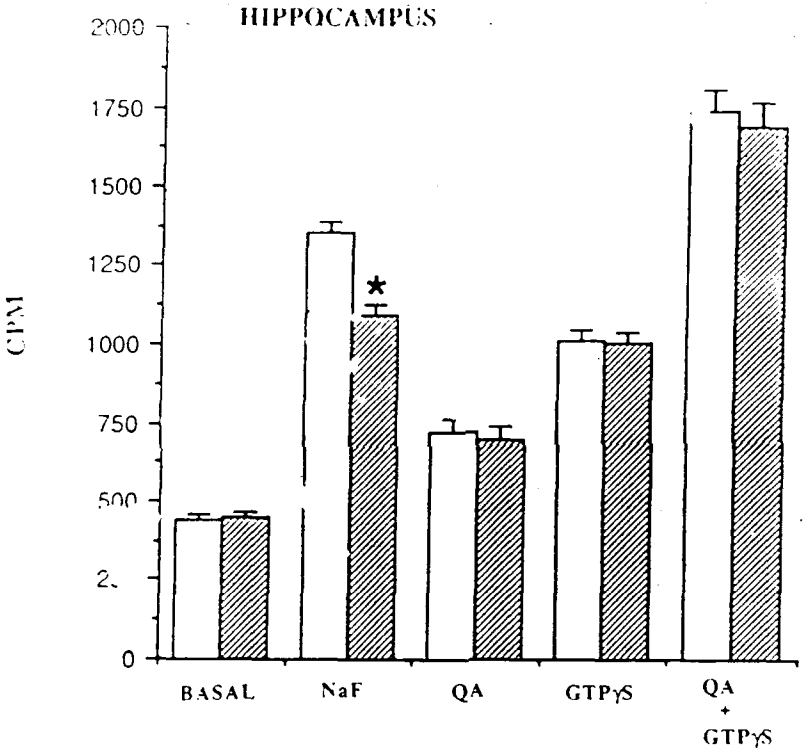


Figure 47. Ca^{2+}-dependent [^{3}H]PI hydrolysis.

Hippocampal membranes were incubated for 20 min at 37° with [^{3}H]PI and the indicated final free Ca^{2+} concentration. Means ± SEM for membranes from 4 control (○) or 4 lithium-treated (●) rats (same groups as those used in Figure 46).

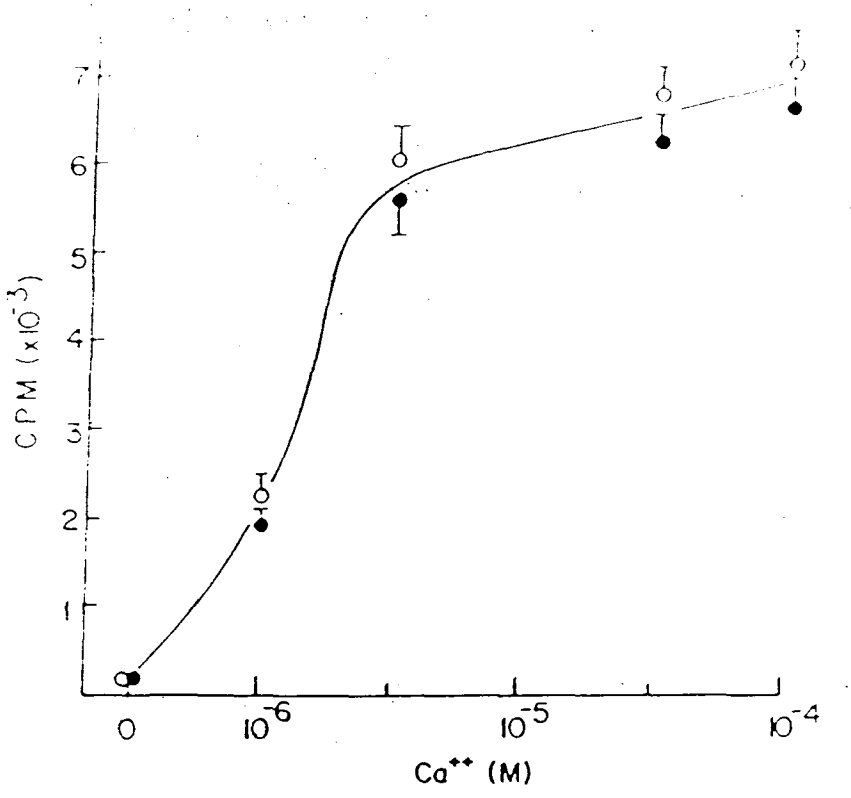


Figure 48. Concentration-response of [^{3}H]PI hydrolysis to (A) GTPγS alone or with carbachol and (B) NaF in cortical membranes from control or acutely lithium-treated rats.

Membranes were incubated with [^{3}H]PI for 20 minutes for 37° with GTPγS (□ control; ■ acute lithium-treated), GTPγS plus 1 mM carbachol (○ control; ● acute lithium-treated), or NaF plus 10 μM $AlCl_3$ (△ control; ▲ acute lithium-treated). Means ± SEM for 3 rats in each group. * $p < 0.05$ compared with controls.

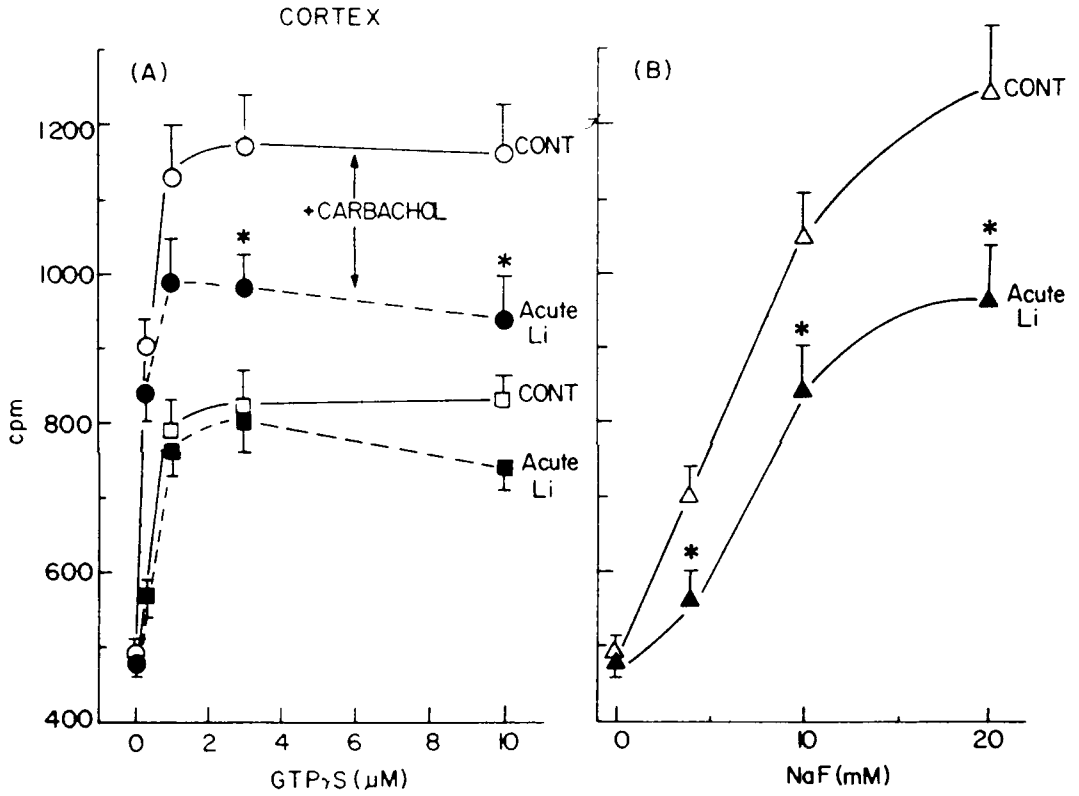


Figure 49. Concentration-response of [^{3}H]PI hydrolysis to (A) GTPγS alone or with carbachol and (B) NaF in hippocampal membranes from control or acutely lithium-treated rats.

Experiments and symbols were described in the legend to Figure 48.

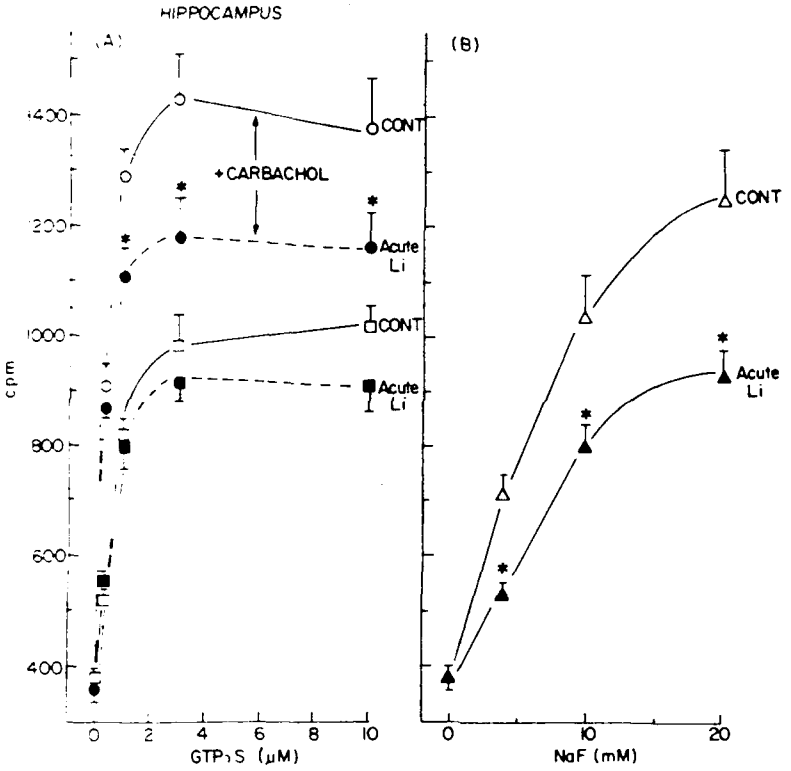


Figure 50. Concentration-response of [^{3}H]PI hydrolysis to (A) GTPγS alone or with carbachol and (B) NaF in striatal membranes from control or acutely lithium-treated rats.

Experiments and symbols were described in the legend to Figure 48.

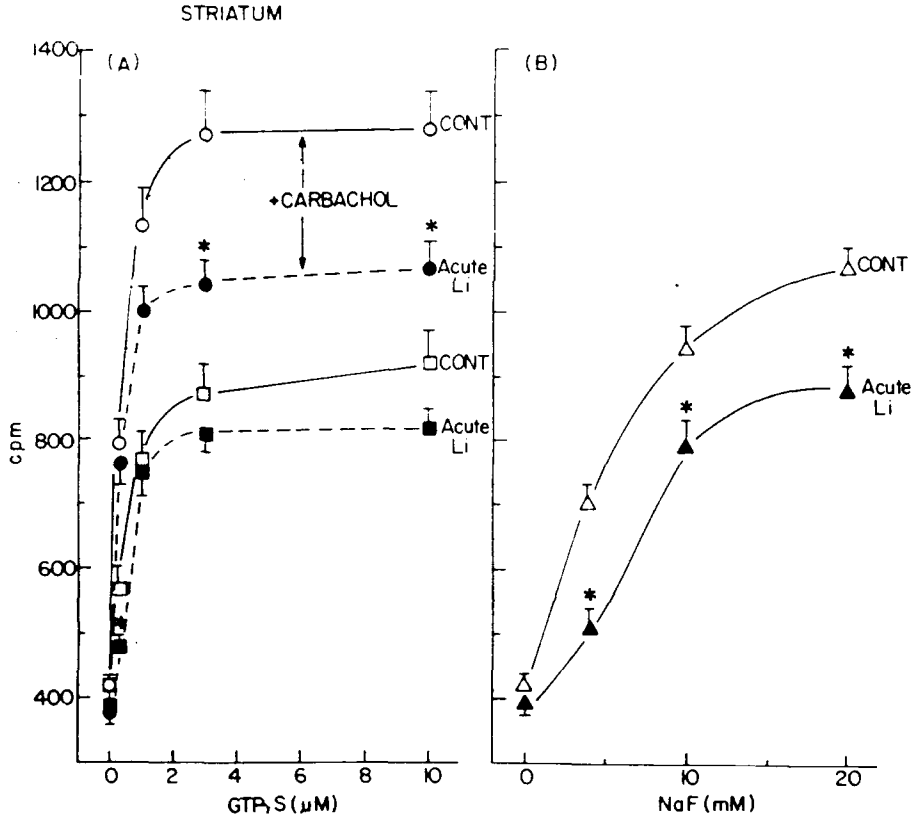


Figure 51. Concentration-response of [^{3}H]PI hydrolysis to (A) GTPγS alone or with carbachol and (B) NaF in cortical membranes from control or seizing rats.

Experiments and symbols were described in the legend to Figure 48.

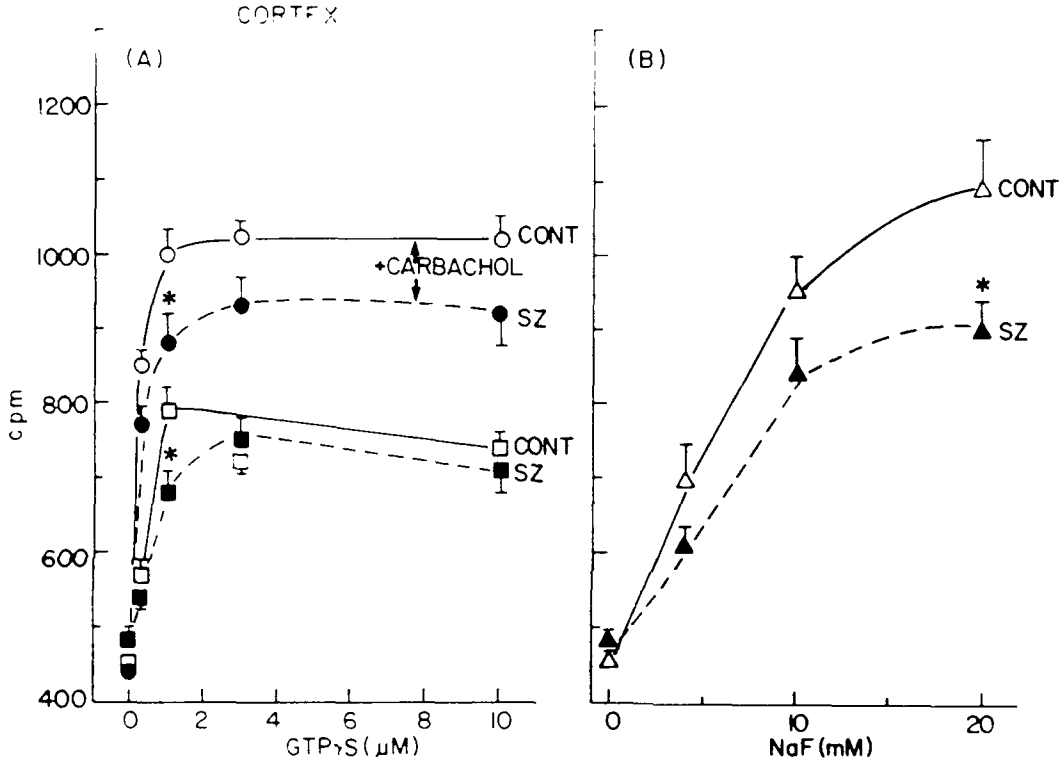


Figure 52. Concentration-response of [^{3}H]PI hydrolysis to (A) GTPγS alone or with carbachol and (B) NaF in hippocampal membranes from control or seizing rats.

Experiments and symbols were described in the legend to Figure 48.

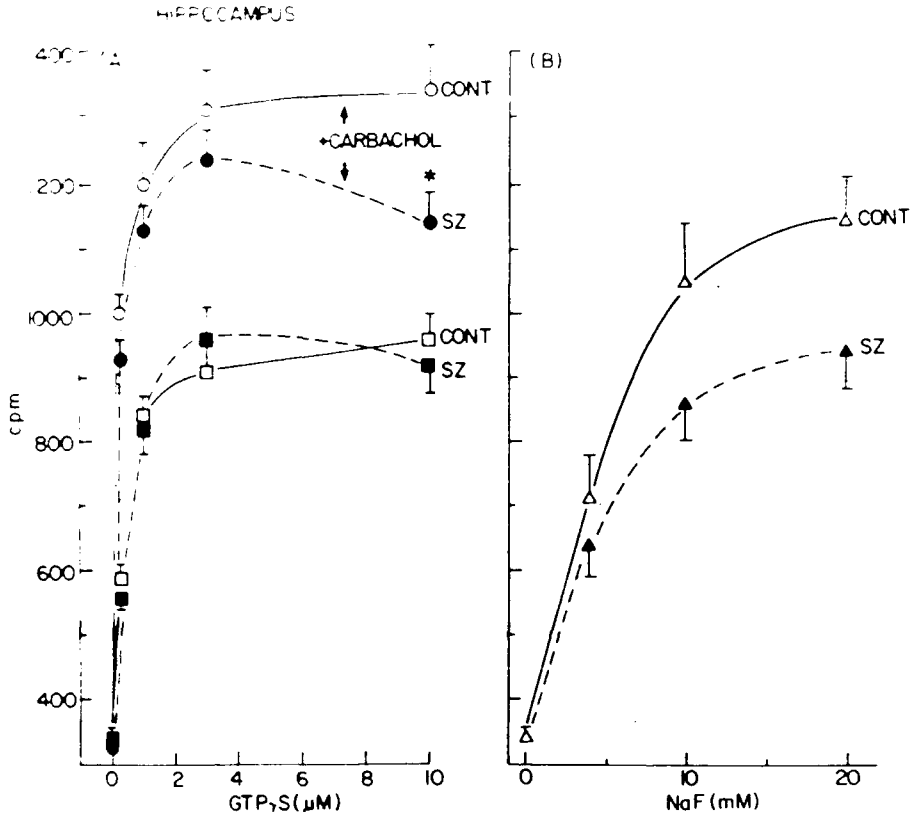


Figure 53. Concentration-response of [^{3}H]PI hydrolysis to (A) GTPγS alone or with carbachol and (B) NaF in striatal membranes from control or seizing rats.

Experiments and symbols were described in the legend to Figure 48.

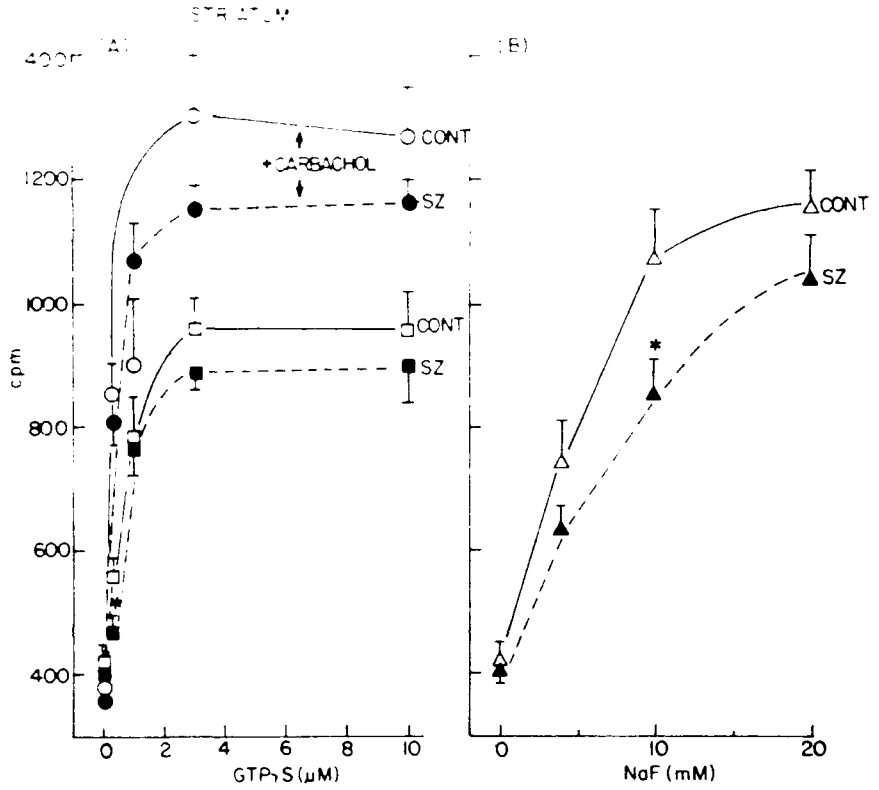


Figure 54. Ins 1,4,5P_3 concentration in rat hippocampus.

Rats were treated with saline (controls, n=28-30), pilocarpine (pilo; 30 mg/kg, sc, 20 minutes (n=10) or 60 minutes (n=7) prior), acute LiCl (3 mmol/kg, ip, 20 hr prior (n=9)), acute LiCl plus pilocarpine (20 min (n=9), or 60 min (n=8) prior), chronic Li (4 weeks (n=11)), or chronic Li plus pilocarpine (20 min (n=10), or 60 min (n=13)). Mean ± SEM. *$p < 0.05$ (ANOVA).

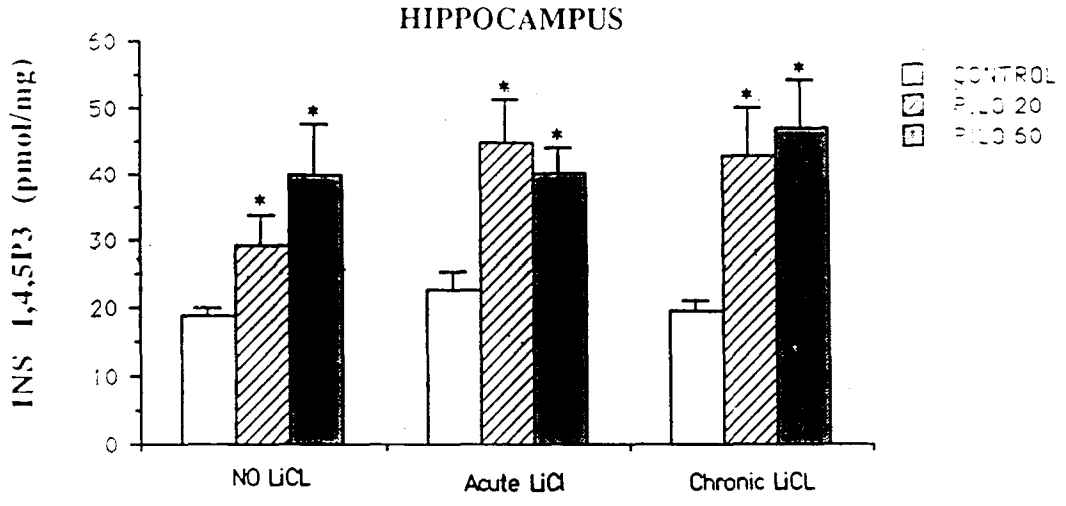


Figure 55. Ins 1,4,5P_3 concentration in rat cerebral cortex.

Experiments were described in the legend to Figure 54.

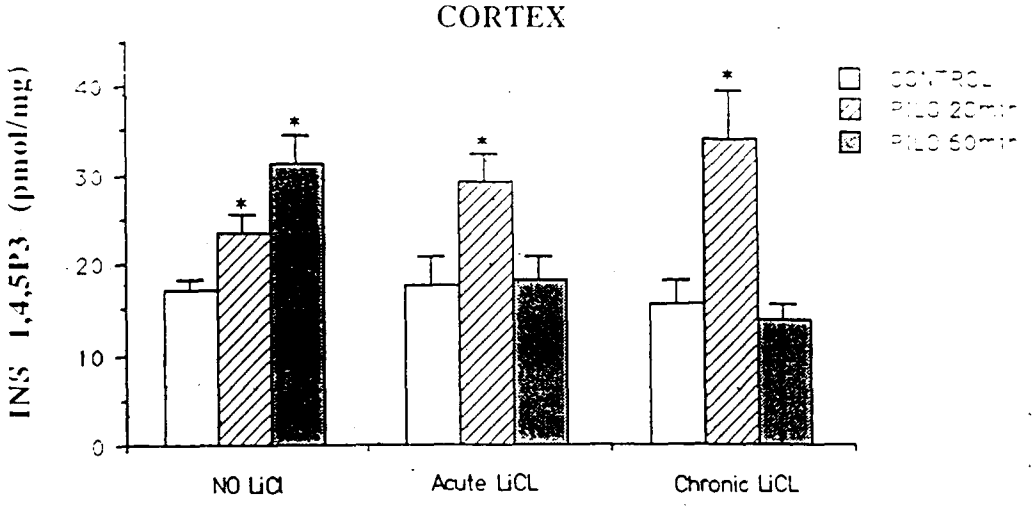


Figure 56. Phosphoinositide hydrolysis in slices from hippocampus (HP) (n=8), cerebral cortex (CTX) (n=12), or striatum (STR) (n=3). Slices were prelabelled with [^{3}H]inositol, incubated with 100 μM ACPD (open bars), 100 μM norepinephrine (hatched bars) or both agents (cross-hatched and filled bars, with the filled part indicating the portion of the response that was greater than additive) for 1 hr in the normal media containing 122 mM NaCl, and [^{3}H]inositol monophosphate ($InsP_1$) was measured as described in the Methods. [^{3}H]$InsP_1$ produced in the absence of agonists was subtracted from the results to obtain the agonist-induced [^{3}H]$InsP_1$ production. Means ± SEM.

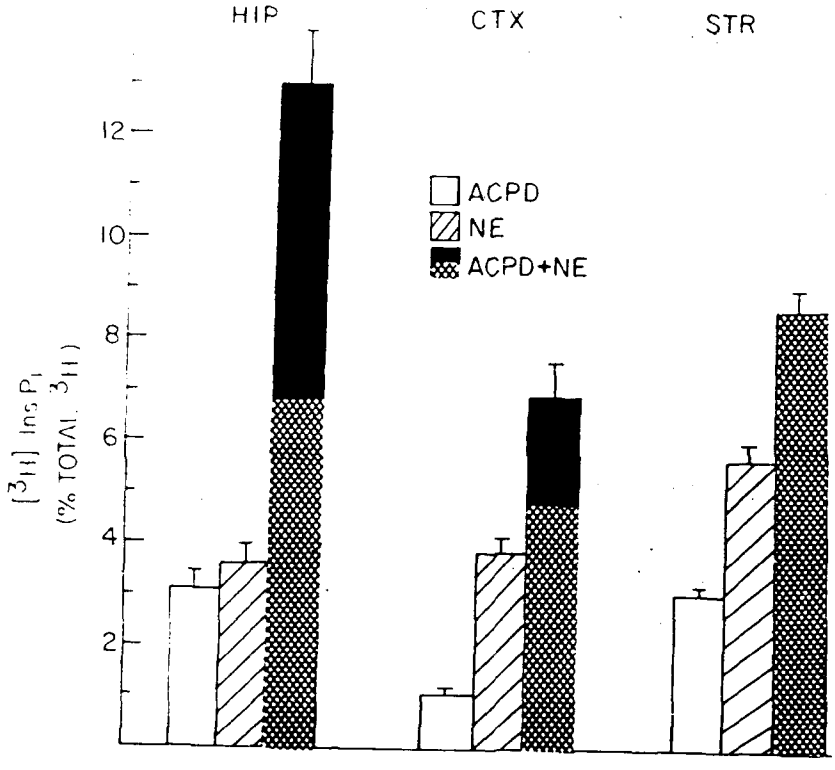


Figure 57. Phosphoinositide hydrolysis in cortical slices incubated with varying concentrations of norepinephrine in the absence (O) or presence (■) of 100 μM ACPD. The theoretical additive values were obtained by adding the [^{3}H]inositol monophosphate ($InsP_1$) produced by each agent alone. [^{3}H]$InsP_1$ produced in the absence of agonists was subtracted from the results to obtain the agonist-induced [^{3}H]$InsP_1$ production. Means ± SEM (n=3).

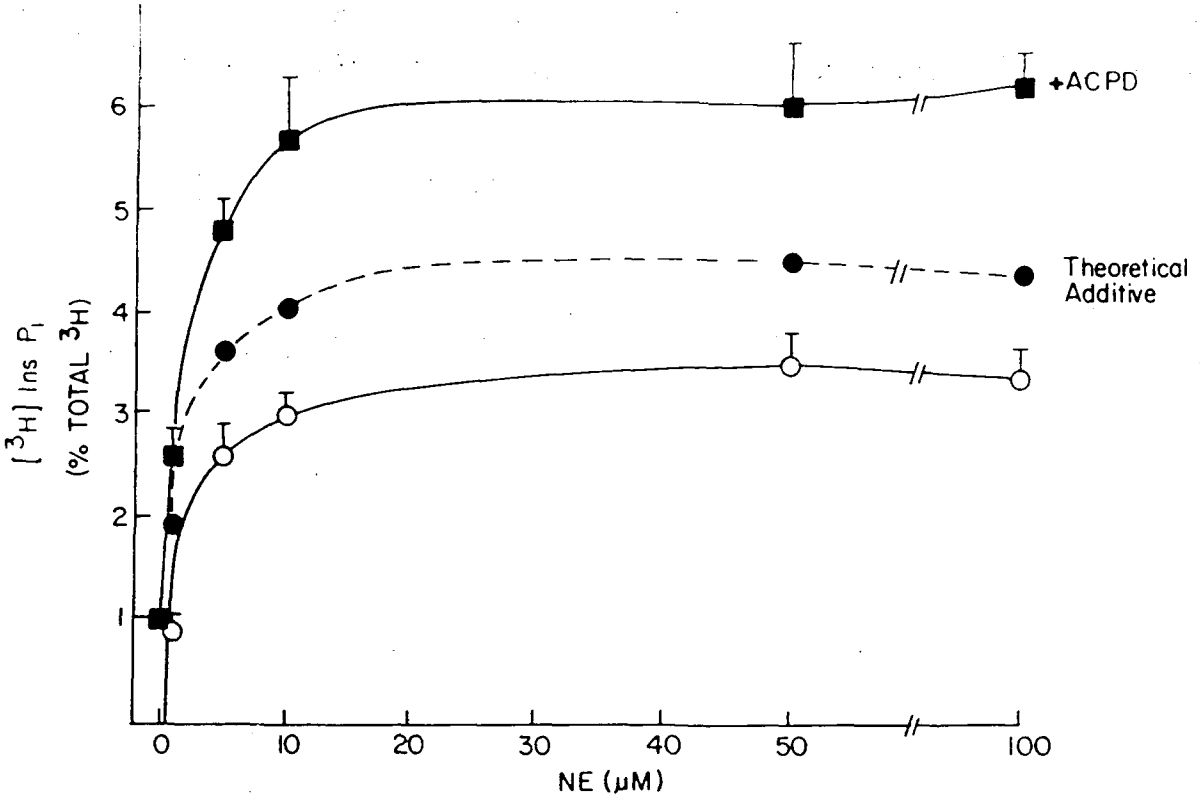


Figure 58. Time course of phosphoinositide hydrolysis in cortical slices incubated with 100 μM ACPD (△), 100 μM norepinephrine (NE) (○), or both agents (■). The theoretical additive values were calculated by adding the [^{3}H]inositol monophosphate ($InsP_1$) produced by each agent alone. [^{3}H]$InsP_1$ produced in the absence of agonists was subtracted from the results to obtain the agonist-induced [^{3}H]$InsP_1$ production. Means ± SEM (n=3).

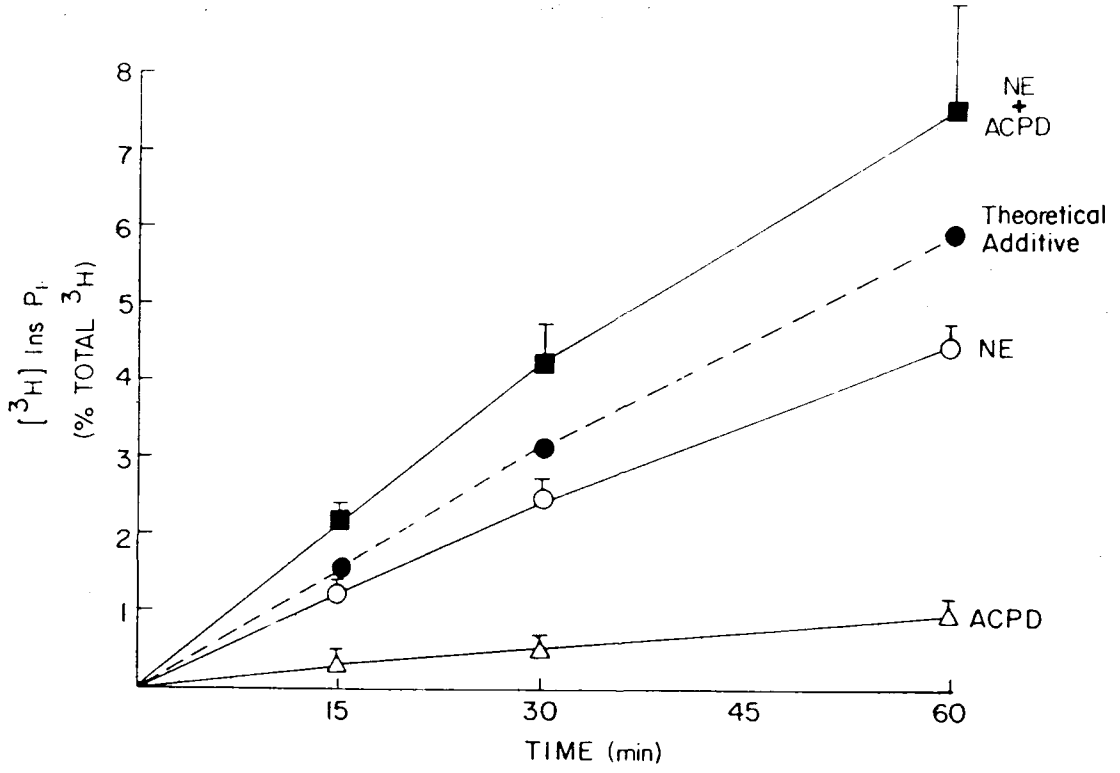


Figure 59. Phosphoinositide hydrolysis in cortical slices incubated with varying concentrations of ACPD in the absence (O) or presence (■) of 100 μM norepinephrine. The theoretical additive values were calculated by adding the [^{3}H]inositol monophosphate ($InsP_1$) produced by each agent alone. [^{3}H]$InsP_1$ produced in the absence of agonists was subtracted from the results to obtain the agonist-induced [^{3}H]$InsP_1$ production. Means ± SEM (n=13 for 100 μM ACPD, n=4-7 for other ACPD concentrations).

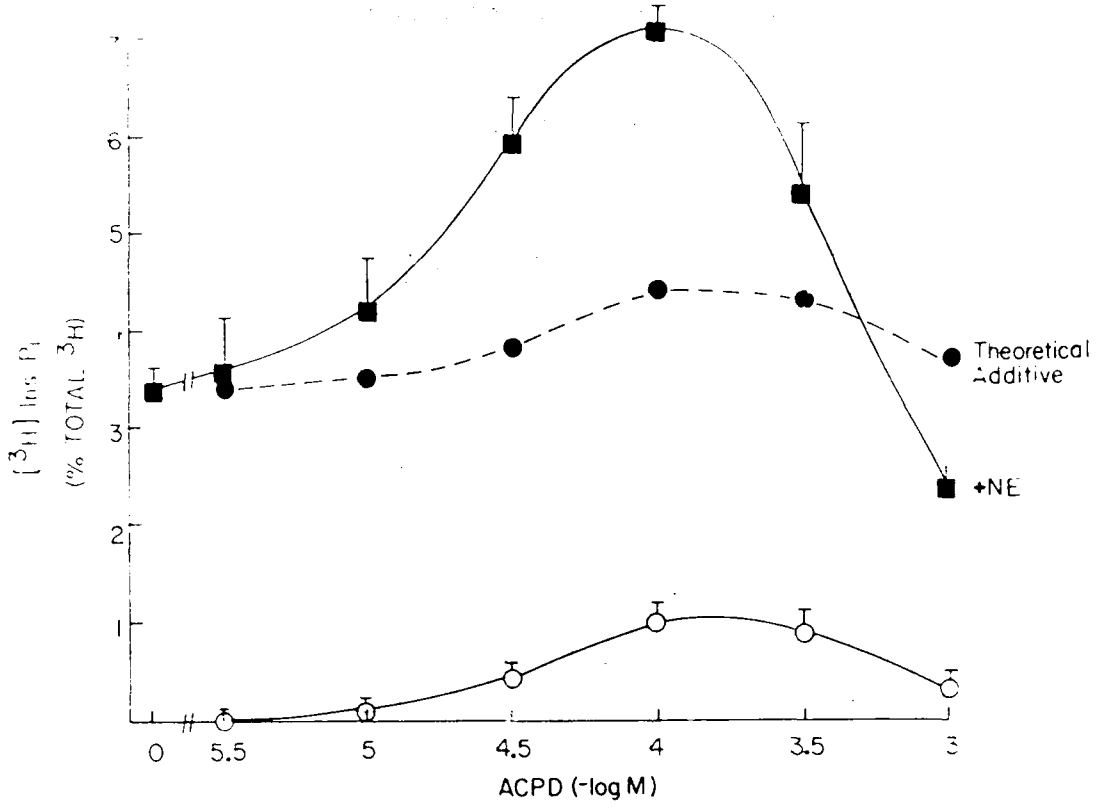


Figure 60. Effects of 10 μM propranolol (hatched bars) and 10 μM prazosin (closed bars) on phosphoinositide hydrolysis in cortical slices stimulated with 100 μM norepinephrine (NE), 100 μM ACPD, or both agonists. Means ± SEM (n=3).

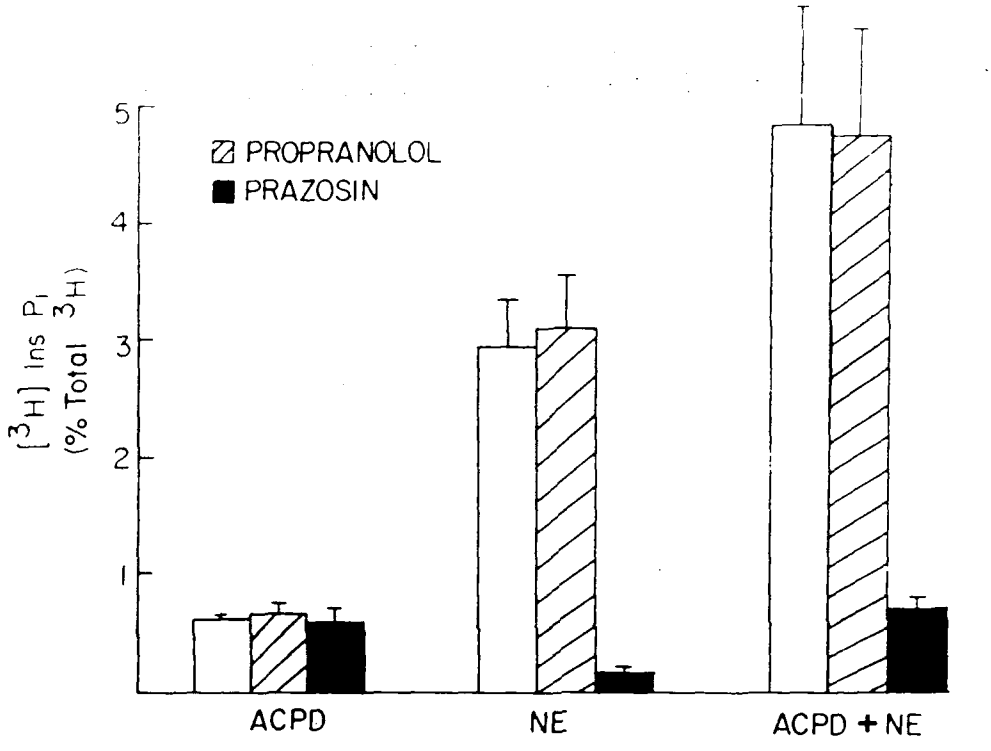
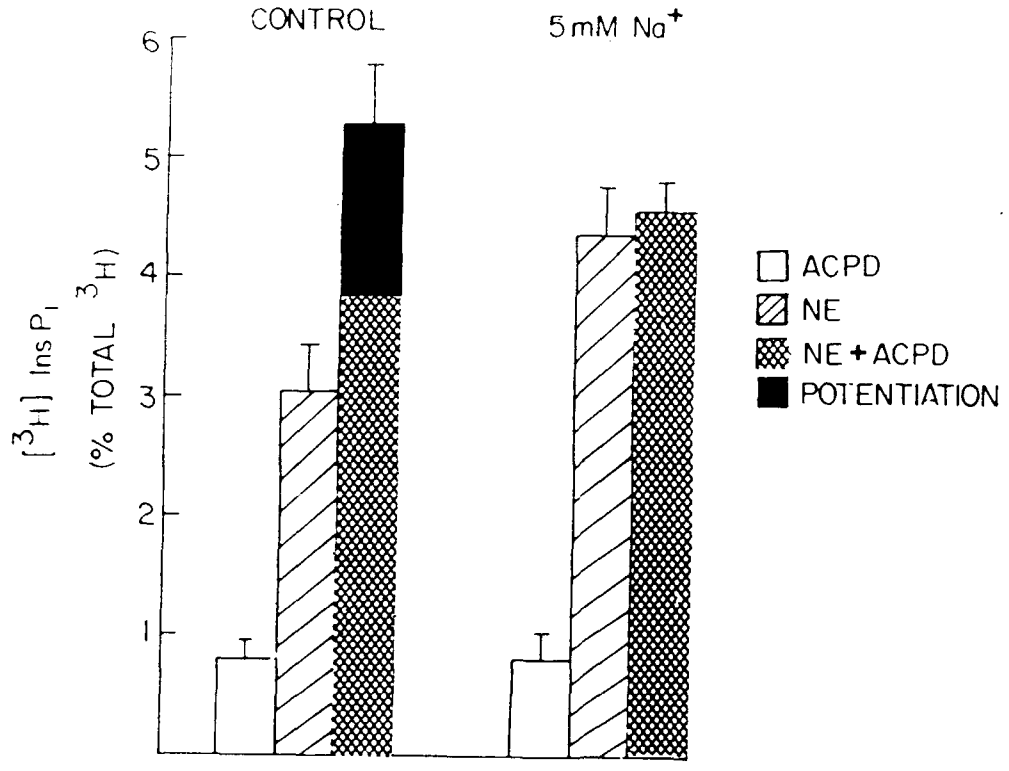


Figure 61. Effect of reduced Na^+ concentration on phosphoinositide hydrolysis. Cortical slices prelabelled with [^{3}H]inositol were incubated in normal media or in media containing 5 mM Na^+ instead of 120 mM Na^+ (NaCl was replaced by choline chloride). The production of [^{3}H]inositol monophosphate ($InsP_1$) induced by 100 μM ACPD (open bars), 100 μM norepinephrine (NE) (hatched bars), or both agonists (cross-hatched and filled bars, with the filled part indicating the portion of the response that was greater than additive) was measured as described in the Methods. [^{3}H]$InsP_1$ produced in the absence of agonists was subtracted from the results to obtain the agonist-induced [^{3}H]$InsP_1$ production. Means ± SEM (n=3).



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PUBLICATIONS

1. Li, X., Song, L. and Jope, R.S. Modulation of phosphoinositide metabolism in rat brain slices by excitatory amino acids, arachidonic acid and GABA. Neurochem. Res.15:731-744 (1990).
2. Ormandy, G.C., Li, X. and Jope, R.S. MK-801 stimulates phosphoinositide hydrolysis in rat cerebral cortical slices. Neuropharmacol. 29:779-782 (1990).
3. Casebolt, T.L. and Jope, R.S. Effects of chronic lithium treatment on protein kinase C and cyclic AMP-dependent protein phosphorylation. Biol. Psychiat. 29:233-243 (1991).
4. Jope, R.S., Li, X., Ormandy, G.C., Song, L. and Williams, M. Reduction of Na+enhances phosphoinositide hydrolysis and differentiates the stimulatory and inhibitory responses to quisqualate in rat brain slices. Brain Res. 536:251-256 (1990).
5. Jope, R.S., Johnson, G.V.W. and Baird, M.S. Seizure-induced protein tyrosine phosphorylation in rat brain regions. Epilepsia 32:755-760 (1991).
6. Jope, R.S., Kolasa, K., Song, L. and Ormandy, G.C. Seizures selectively impair agonist-stimulated phosphoinositide hydrolysis without affecting protein kinase C activity in rat brain. Neurotoxicology 13:389-400 (1992).
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8. Jope, R.S., Song, L. and Kolasa, K. Inositol trisphosphate, cyclic AMP and cyclic GMP in rat brain regions after lithium and seizures. Biol. Psychiat. 31:505-514 (1992).
9. Kolasa, K. and Jope, R.S. Synergistic activation of phosphoinositide hydrolysis induced in brain slices by norepinephrine and the excitatory amino acid agonist trans-ACPD. Neuropharmacology (in press).

MEETING ABSTRACTS

1. Ormandy, G.C., Li, X, Song, L., Williams, M.B. and Jope, R.S. Alterations of Na^+ differentially affect agonist-induced phosphoinositide hydrolysis in rat brain slices. Soc. Neurosci. 16:1300 (1990).
2. Li, X., Song, L., and Jope, R.S. Phosphatidylinositol hydrolysis in brain membranes. Soc. Neurosci. 17:1605 (1991).
3. Baird, M.S., Jope, R.S. and Johnson, G.V.W. Seizure-induced protein tyrosine phosphorylation in rat brain regions. Soc. Neurosci. 17:1605 (1991).
4. Faulk, A.G., Song, L., Kolasa, K. and Jope, R.S. IP_3, cyclic AMP, and cyclic GMP in rat brain regions after lithium and seizures. Soc. Neurosci. 17:1606 (1991).

PERSONNEL SUPPORTED

1. Richard S. Jope, PI
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GRADUATE DEGREES

1. Xiaohua Li, Ph.D. in Pharmacology from The University of Alabama at Birmingham, March, 1991.